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Poor Maternal Nutrition Reduced Body Weight, Growth Variables, and Circulating Concentrations of IGF-I and IGFBP-3 in Lambs

Melissa A. Rokosa
University of Connecticut - Storrs, MRokosa17@gmail.com

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Melissa Anne Rokosa

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Poor Maternal Nutrition Reduced Body Weight, Growth Variables, and Circulating Concentrations of IGF-I and IGFBP-3 in Lambs

Presented by

Melissa Anne Rokosa, B.S

Major Advisor_____
Dr. Steven A. Zinn

Associate Advisor_____
Dr. Kristen E. Govoni

Associate Advisor_____
Dr. Thomas A. Hoagland

Associate Advisor_____
Dr. Sarah A. Reed

University of Connecticut

2013

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TABLE OF CONTENTS

LIST OF TABLES

TABLES **PAGE**

LIST OF FIGURES

FIGURES **PAGE**

ABBREVIATIONS

AU	Arbitrary unit
BCS	Body condition score
BMC	Bone mineral content
BMD	Bone mineral density
bGH	Bovine growth hormone
cAMP	Cyclic adenosine monophosphate
CS	Chorionic somatomammotrophin
Con-lamb	Lambs born to control-fed ewes
CP	Crude protein
DLU	Digital light unit
DEXA	Dual X-Ray absorptiometry analysis
GHBP	Growth hormone binding protein
GH	Growth hormone
GHR	Growth hormone receptor
GHRH	Growth hormone releasing hormone
IGF (-I/-II)	Insulin-like growth factor (-I/-II)
IGFBP	Insulin-like growth factor binding protein (1,2,3,4,5,6)
IUGR	Intrauterine growth retardation
mRNA	Messenger ribonucleic acid
NRC	National research council
Over-lamb	Lambs born to overfed ewes
oGH	Ovine growth hormone
pGH	Porcine growth hormone
PL	Placental lactogen
PKA	Protein kinase A pathway
RIA	Radioimmunoassay
rGH	Recombinant growth hormone
rhGH	Recombinant human growth hormone
Res-lamb	Lambs born to restricted-fed ewes
SAS	Statistical analysis system
SRIF	Somatostatin
T3	Triiodothyronine
T4	Thyroxine
TC	Total cholesterol
TDN	Total digestible nutrients
TG	Triglycerides
USDA	United States Department of Agriculture

Introduction

Growth is a coordinated process in which new cells are formed through cell division while existing cells increase in size; as cells grow and develop they concurrently specialize to a specific cell lineage and purpose (Goranov et al., 2009). These processes of growth and development are mediated through numerous endocrine pathways, one of the most critical being the somatotropic axis. The main components of the somatotropic axis are the hypothalamus, anterior pituitary gland, the liver and peripheral tissues (e.g., adipose, muscle, and bone). When functioning normally, the hormones and binding proteins of the somatotropic axis interact with other hormonal systems that also have a role in growth regulation. These additional systems include thyroid hormones, glucocorticoids, leptin, testosterone, estrogen, insulin and locally produced growth factors (Brier et al., 1999). However, the somatotropic axis can be disrupted, due to changes in nutritional status, disease state, age and homeostasis (Redman and Ravussin, 2008). These changes will impact the somatotropic axis, thyroid hormones, glucocorticoids, sex hormones as well as other hormones. This study focused upon the relationship between the somatotropic axis, maternal nutrition, and the effects on lambs during neonatal and postnatal growth and development.

Intrauterine growth retardation is defined as the impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy (Wu et al., 2006). There are numerous aspects of growth and development that can be affected in instances of intrauterine growth retardation (IUGR), including but not limited to pre-weaning survival, reproductive performance, postnatal growth, body composition, and meat quality (Wu et al., 2006). One extrinsic factor that has an impact on the

somatotropic axis is the effect of maternal nutrition on the postnatal growth of the progeny. Poor maternal nutrition can include under-nutrition or over-nutrition of an animal in terms of energy, protein, and/or minerals. Both can increase the instances of fetal death and can cause reduced fetal growth (Wu et al., 2006). This literature review will focus on the components of the somatotropic axis, followed by a review of the interaction of the somatotropic axis with different tissues, including adipose, bone, muscle, and then conclude with a consideration of the impact of IUGR on growth and development, and its connection to the somatotropic axis.

Literature Review

Somatotropic Axis

During postnatal growth, multiple growth factors work in conjunction with the somatotropic axis to promote the accretion of protein and positive energy balance (Breier et al., 1999). The somatotropic axis is one of the key modulators of postnatal growth. The somatotropic axis consists of the hypothalamus and the anterior pituitary gland, which are connected via the hypothalamic portal blood system (Brown-Borg, 2009). Beyond the hypothalamic portal blood system, the liver, bone, muscle, and adipose tissue are downstream components of the somatotropic axis (Brown-Borg, 2009). The hormones that are part of the axis are growth hormone releasing hormone (GHRH), somatostatin (SRIF), growth hormone (GH), insulin-like growth factor (IGF) I and II and IGF binding proteins (IGFBP).

Growth Hormone

One of the major hormones of the somatotropic axis is GH. Growth hormone is synthesized, secreted, and stored by the somatotrope cells of the anterior pituitary gland (Tuggle and Trenkle, 1995; Cuttler, 1996). The types of GH stored within the somatotrope is dependent upon the molecular weight, the timing of GH synthesis, and additional outside stimuli (Cuttler, 1996). Growth hormone is normally released in a pulsatile manner (Tuggle and Trenkle, 1995), and changes in pulsatility are caused by the interaction of two hypothalamic hormones, SRIF and GHRH. Growth hormone

releasing hormone stimulates the secretion and synthesis of GH, while simultaneously SRIF inhibits GH through inhibiting the actions of GHRH (Cuttler, 1996).

Growth Hormone belongs to the superfamily of cytokine hormones (Flint et al., 2003); this family also includes placental lactogens and prolactins (Kopchick, 2003). It generally can be classified as having anabolic effects by modifying homeostasis within the body (Castellano et al., 2009). Approximately, 350 million years ago, the GH gene evolved from duplication of an ancestral gene (Kopchick, 2003). There are four gene clusters that produce variants of GH; GH1 produced from somatotrope cells, GH2 which is produced at the placenta, and two gene clusters that produce chorionic somatomammotrophin (CS) -1 and -2, also known as placental lactogen (PL) additionally produced by the placenta (Baumann, 2009). The gene for GH is on the long arm of the 17th chromosome in humans and is surrounded by a cluster of closely related genes (Kopchick et al., 2002). In cattle, the locus is located on the 19th chromosome, and in sheep, it is located on the 11th chromosome (Hediger et al., 1990). The precursor amino acid is derived from a sequence of five exons and four introns and produces a peptide that is 217 amino acids in length (Kopchick et al., 2002). Due to proteolytic cleavage of the amino-terminal signal peptide, a final single-chain polypeptide of 191 amino acids in length with two disulfide bonds, is produced (Kopchick et al., 2002; Baumann, 2009). The majority of GH in circulation has a molecular weight of approximately 22,129 daltons (22K-GH). This represents 70 to 75% of circulating GH, whereas the 20,000 dalton (20K-GH) form represents 5 to 10% of circulating GH in humans (Tuggle and Trenkle, 1995). The 20K-GH form is created by alternative splicing at the third exon of the precursor messenger ribonucleic acid (mRNA), and it is also

produced by the anterior pituitary gland (Kopchick et al., 2002; Baumann, 2009). In sheep, there are two GH genes, ovine (o) GH-1 and oGH-2; oGH-2 expressed in the placenta has two alleles, oGH-2N and oGH-2Z (Wright et al., 2008). Wright et al. (2008) reported that oGH-2 is only expressed by the placenta during a specific time frame, with increased expression beginning at day 35, peaking at day 45, and ending at day 55. The exact function of this oGH-2 peak has not been deciphered and still requires additional research. The crystalline structure of porcine (p) GH was reported by Abdel-Meguid et al (1987); they showed that the pGH molecule contains four alpha-helical bundles, with two disulfide bridges in an antiparallel topography. Bovine (b) GH and oGH are similar to pGH, both contain two disulfide bridges and one tryptophan residue in each molecule (Li and Yang, 1974; Santome et al., 1966). In conclusion, the GH molecule is highly conserved, with characteristics that make each form species specific.

Secretion and Regulation

There are many factors that influence the control and secretion of GH from the somatotrope cells. Proliferation and differentiation of somatotropes are mainly induced by GHRH binding with its receptor on somatotropes (Weigent, 1996). This activation of somatotrope cells causes an increase in intracellular cyclic adenosine monophosphate (cAMP) concentrations; which impacts GH synthesis (Weigent, 1996). In addition, the synthesis and secretion of GH from somatotropes are stimulated by increased intracellular Ca^{2+} concentrations (Chang et al., 2012). Concentrations of Ca^{2+} are linked to changes in membrane potention, phosphorylation of cAMP-dependent proteins, and

phosphorylation of PKC-dependent proteins (Lussier et al., 1991). Under basal conditions, when the voltage-sensitive ionic channels of a somatotrope are activated, intracellular Ca^{2+} concentrations increase as Na^{+} enters the cell (Chang et al., 2012). Lussier et al. (1991) reported that GHRH-stimulated membrane depolarization increases Ca^{2+} concentrations, which were then sustained by the presence of the protein kinase A (PKA) pathway. When SRIF is present there is decreased intracellular Ca^{2+} concentrations due to a hyperpolarization of the membrane that prevents voltage sensitive Ca^{2+} channels from functioning (Lussier et al., 1991).

The secretion of GH is both pulsatile and circadian (ThidarMyint et al., 2008). The pulsatility of GH is mainly controlled by the hypothalamic hormones GHRH and SRIF (Nass et al., 2011). Growth hormone is secreted consistently at a basal concentration, with GHRH stimulating increased concentrations (Nass et al., 2011). Muller et al. (1999) reported the link between the pulsatile release of GHRH and the pulsatile release of GH. Although the majority of GH pulses are linked with the release of GHRH from the anterior pituitary gland, Frohman et al. (1990) showed that 30% of the pulses did not coincide. There is a possibility that another hypothalamic factor other than GHRH and SRIF is responsible for the pulses that do not coincide with the GHRH release (Frohman et al., 1990). Somatostatin has an inhibitory effect upon GHRH, resulting in decreased pulse intensity of GH released (Frohman et al., 1990).

Following secretion from the anterior pituitary gland, the majority of the GH is bound to GH binding protein (GHBP; Cuttler, 1996). This binding protein corresponds with the extracellular domain on the GH receptor in the liver (Renaville et al., 2002). There are two forms of the binding protein, a high-affinity form and a low affinity form

(Cuttler, 1996). The concentrations of the GHBP have no correlation with spontaneous GH secretion, but are correlated with basal concentrations (Cuttler, 1996). In normally growing boys, there is a correlation between body mass index and serum concentration of the high-affinity GHBP (Argente et al., 1996). The correlation between these two factors allowed researchers an opportunity to use GHBP as a marker of growth disorders (Argente et al., 1996).

After GH is secreted from the anterior pituitary gland and binds to the GHBP, the GH complex enters the general circulation, where it can have either direct effects on tissues or indirect effects via IGF-I (Holt, 2002). Additional effects of GH are mediated through IGF-I, which is secreted from target tissues and is discussed below. The direct effects of GH are mainly as a promoter of linear growth and the metabolism of target tissues (e.g., muscle, adipose and bone; Cuttler, 1996). Growth hormone has also been linked with changes in lipid, nitrogen, carbohydrate and mineral metabolism (Kopchick et al., 2002). Other activities that are influenced by GH include the ability to regulate the differentiation of preadipocytes into adipocytes, brain and cardiac function, and development of immune function (Morikawa et al., 1982; Chen et al., 1998). The biological actions of GH are mediated through the binding to its specific cell-surface receptor; the GH receptor (GHR; Kopchick et al., 2002).

Growth Hormone and Adipose Tissue

Growth hormone plays an integral role in the development of numerous organ systems and tissues, including adipose tissue. Two important process in relation to adipose tissue are adipogenesis and lipolysis. Adipogenesis is the process in which pre-adipocytes are committed to the adipocyte cell lineage (Moller and Jorgensen, 2009). Lipolysis is the breakdown of lipids into free fatty acids (Moller and Jorgensen, 2009). Growth hormone plays an important role in the increase in adipocyte cell size; humans treated with exogenous GH reduced adipocyte size and number (Brook, 1973). Adipose tissue does not express GH, but it does express IGF-I at the adipocyte and pre-adipocyte stages (Bluher et al., 2005). The fat mass of an animal is determined by the balance between the synthesis and catabolism of lipids (Haemmerle et al., 2006). One direct action of GH is to increase lipolysis, as well as to increase lipid oxidation through improvements in substrate availability (Bluher et al., 2005). The differentiation of pre-adipocytes to adipocytes is highly controlled by the actions of IGF-I, which is stimulated by GH (Bluher et al., 2005). When exogenous pGH was administered, it was reported that cells had an altered response to insulin, resulting in changes in the ability of the cell to undergo lipogenesis (Flint et al., 2003). This reduction in insulin sensitivity, in turn, decreased expression of lipogenic enzyme genes and glucose transport (Flint et al., 2003). When an animal is experiencing nutrient restriction, the resulting increase in GH will inhibit adipogenesis or stimulate lipolysis (Gregoire et al., 1998; Poulos et al., 2010). Growth hormone deficient humans will as a result of the deficiency have an associated increase in fat mass (Berryman et al., 2011). This deficiency can be treated using

recombinant human GH (rhGH), which will cause a decrease in fat mass, but as soon as the treatment ends the fat mass will increase again (Berryman et al., 2011).

Growth Hormone and Muscle Tissue

In conjunction with its effects upon adipose tissue, GH also regulates muscle tissue and therefore carcass composition. Growth hormone acts to increase muscle protein synthesis (Urban, 1999). Growth hormone deficient humans have a reduction of lean muscle mass of 7 to 8% compared with individuals producing normal concentrations of GH (Lissett and Shalet, 2000). As an individual ages the production of GH from the anterior pituitary gland decreases, resulting in an associated increase in fat mass and decreased lean body mass (Urban, 1999). Injection of exogenous GH has been one treatment used to offset the loss of naturally produced GH (Aroniadou-Anderjaska et al., 1996). Growth hormone increases growth rate and muscle mass in many species. For example in rats, the use of recombinant GH (rGH) correlated with an accelerated accretion of body weight gain as well as a proportional increase in muscle growth (Aroniadou-Anderjaska et al., 1996). In human patients that were given an infusion of GH into the forearm, there was a net increase in protein anabolism and protein synthesis at the injection location (Fryburg and Barrett, 1993). Although there was increased skeletal muscle mass, whole-body protein metabolism was unaffected by the GH infusion (Fryburg and Barrett, 1993). In a study using pigs, exogenous GH administration increased production efficiency by 15 to 35%, growth rate by 10 to 20%, and protein deposition increased by up to 50% (Etherton et al., 1993). These changes in

carcass composition associated with exogenous GH administration increased whole-body muscle mass while decreasing adipose tissue accretion (Etherton et al., 1993). In sheep, exogenous GH treatment of 2.5 mg for 50 days resulted in an increase in body weight of 2.2 kg compared with controls (Heird and Hallford, 1991). The increased body weight gain is associated with an increase in feed efficiency in the sheep treated with GH; that is, despite the increase in body weight gain there is a decreased feed intake of 0.9 kg (Heird and Hallford, 1991).

Alterations in Growth Hormone Secretion

Growth hormone deficiency causes numerous adverse effects on growth and metabolism. Circulating concentration of GH are typically increased when animals are receiving a poor diet or are undernourished as an adult (Breier, 1999). In the case of a growing animal that has undergone prolonged fasting, there is increased circulating GH (Breier, 1999). A similar trend was also observed in dairy cows in the first week post calving (Renaville et al., 2002). This increase in GH is due to a reduction in GH clearance in undernourished animals or when cows are in a negative energy balance (Breier, 1999). Due to decreased clearance of GH, the feed-back mechanism to SRIF and the interaction at the GH receptor is altered (Breier, 1999). Additionally increased GH stimulates IGF secretion, promotes lipolysis, increases protein synethsis, and has a role in maintain homeostasis (Breier, 1999). Increased protein intake resulted in a subsequent increase in frequency of GH pulses (Renaville et al., 2002). Infants between 3 and 15 months of age that experienced malnutrition had increased baseline

concentrations of GH [$\bar{x}$ = 18.3 ng/mL of GH] depending upon stage of malnutrition, compared with normal infants that averaged 11.8 ng/mL (Muzzo et al., 1990). Growth hormone supplementation is being explored as a method to achieve optimal nutrition and growth for cystic fibrosis patients (Hardin et al., 2005). Hardin et al. (2005) reported that patients given GH for one year as an adjunct to nutritional supplementation had improved protein kinetics, increased IGF-I concentrations, increased bone mineral content, and improved pulmonary function.

Growth Hormone-Releasing Hormone

The hypothalamic hormone responsible for stimulating the secretion and synthesis of GH from the somatotrope cells is GHRH (Cuttler, 1996). The hypothalamic cells that produce GHRH in rats and humans are located in the mediobasal part of the hypothalamus (Cuttler, 1996).

Growth hormone releasing hormone was first isolated in human pancreatic tumor cells and was determined to be a 44 amino acid poly-peptide (Brazeau et al., 1982). It was then linked to the hypothalamus as its main production site in both humans and animals (Brazeau et al., 1982). The gene for GHRH in humans is 10 kilobases long and contains five exons (Frohman et al., 1989), it is located on the 20th chromosome (Cuttler, 1996). This coding region produces two nearly identical mRNA, which only differ from each other due to a difference in splicing site on the fifth exon (Frohman et al., 1989). The mRNA is then used to create an initial peptide of either 108 or 107 amino acids, which is then spliced into the final active 44 amino acid form (Frohman et al.,

1989). Ovine and caprine GHRH are also an 44 amino acid peptide (Brazeau et al., 1984). Both sequences are nearly identical to the bovine GHRH, and the main difference between them is a replacement at position 13 of an isoleucine with a valine (Brazeau et al., 1984). In bovine and porcine, GHRH is similar to the human analog, with differences of 5 and 3 amino acids within the sequence respectively (Brazeau et al., 1984).

Growth hormone releasing hormone is part of the hormone family that includes pituitary adenylate cyclase-activating peptide, secretin, vasoactive intestinal peptide, glucagon, glucagon-like peptides (-1 & -2), and gastric inhibitory peptide (Barabutis and Schally, 2010). This hormone family increases the concentration of intracellular cAMP, which in turn activates the PKA (Barabutis and Schally, 2010). The PKA pathway is a cAMP-dependent pathway in which cAMP activates the PKA enzyme which phosphorylates a variety of enzymes (Walsh et al., 1994). The receptor for GHRH is located on the anterior pituitary gland, and is classified as a G-protein-coupled receptor. Porcine GHRH receptor is homologous with the receptors for pituitary adenylate cyclase activating peptide and vasoactive intestinal peptide, all of which have seven transmembrane domains (Barabutis and Schally, 2010). In addition to its role in increasing intracellular calcium, GHRH stimulates the secretion of GH from the somatotrope (Tuggle and Trenkle, 1995). In mice over-expressing GHRH, or in humans with tumors that overproduce GHRH, there is hyperplasia of the somatotrope cells, which in some cases can result in pituitary adenoma (Pombo et al., 2001). Alternative mutation in the GHRH receptor results in hypoplasia of somatotropes and impairs GH secretion (Pombo et al., 2001). The biological action of GHRH, is highly reliant on the

tyrosine and histidine residues that are part of the amino terminal region of the peptide; these are essential for correct binding to GHRH receptor (Frohman et al., 1989). Sheep have on average of 4.2 pulses of GHRH over a five-hour period, meantime between pulses is 71 minutes with the peak concentrations ranging from 25 to 40 pg/mL (Frohman et al., 1990).

Somatostatin

The hypothalamic hormone responsible for suppressing the secretion and synthesis of GH from the anterior pituitary gland is SRIF. The mature form of SRIF is either a 14- or 28- amino acid peptide synthesized from a preliminary 116 amino acid that is cleaved to its smaller active forms (Cuttler, 1996). The active forms of SRIF are released into the hypothalamic portal blood system in a pulsatile manner in different proportions within the same neurons and secretory cells (Tuggle and Trenkle, 1995). The 28-SRIF is an amino-terminal extension of the 14-SRIF version, but the SRIF-28 has a superior ability to suppress GH secretion (Tuggle and Trenkle, 1995). As reported by Frohman et al. (1990) using ovariectomized mature Corriedale ewes, average concentrations of SIRF were between 65 and 160 pg/mL with an average time between pulses of 52 minutes. Growth hormone concentrations can be repressed directly through the actions of SRIF or indirectly through blocking the actions of GHRH (Tuggle and Trenkle, 1995). The receptor for somatostatin is a 7 transmembrane domain glycoprotein that has 7 membrane spanning alpha-helical domains (Bokum et al., 2000). Somatostatin is also produced by hepatic tissue, and secreted from the D cells from the

pancreas and gastrointestinal tract (Bokum et al., 2000). In peripheral tissues, somatostatin inhibits secretion of gastrin, glucagon, insulin, calcitonin, and vasoactive intestinal peptides (Bokum et al., 2000). Growth hormone releasing hormone and SRIF are antagonistic to each other in an ultra-short feed-back loop, which controls the release of GH (Wankowska et al., 2012). Growth hormone represses its own secretion through a short-loop feed-back system that inhibits GHRH release from the hypothalamus and subsequently stimulates SRIF (Wankowska et al., 2012).

Insulin-like Growth Factor I and II

Two of the primary hormones of the somatotropic axis are IGF-I and IGF-II. Insulin-like growth factor is primarily a mediator of the growth effects of GH (Baker et al., 1993). There are two different IGF compounds, IGF-I and IGF-II, and each influences growth at distinctively different time points (Setia and Sridhar, 2009). Insulin-like growth factor-I is the central mediator of postnatal growth, whereas IGF-II is the central mediator of prenatal growth (Baker et al., 1993). Both IGF-I and IGF-II share 70% homology with each other, 40% homology with insulin (Hill, 1996) and 50% homology with proinsulin (Renaville et al., 2002). Both forms of IGF have an A and a B chain that are connected by a C peptide; to increase the mitogenic potency IGF, they contain a D peptide extension at the carboxy-terminus (Hill, 1996). The molecular weight of IGF is approximately 7,600 Daltons (Hill, 1996). Insulin-like growth factor -I and IGF-II have 70 and 67 amino acids, respectively (McGuire et al., 1992). Honegger and Humbel (1986) reported that IGF-I isolated from bovine serum was identical in amino acid structure to

IGF-I isolated from human serum (McGuire et al., 1992). Insulin-like growth factor-II differs between species, whereas the amino acid sequence of IGF-I are highly conserved over a variety of additional species (Renaville et al., 2002). Exogenous IGF-I is administered to lambs it results in reduction of protein catabolism and stimulation of protein metabolism (Breier, 1999). The treatment of exogenous IGF-I to the point of saturating the binding proteins, all proteins bound, glucose metabolism is altered; in lambs it increases glucose concentrations, while suppressing insulin release (Breier, 1999). Insulin-like growth factor is mainly produced when GH binds with its receptor on the hepatic cells, but it can also be produced locally by specific organs and tissues (Collett-Solberg and Cohen, 1996). Insulin-like growth factor that is produced from peripheral tissues primarily has an autocrine or paracrine role (Hill, 1996).

Insulin-like Growth Factor, Embryogenesis and Early Postnatal Growth

During development, the expression and distribution of the IGF vary depending on the stage of growth or development (Hill, 1996). In the mouse embryo, transcription of mRNA for IGF begins when the embryonic genome takes over from maternal control (Hill, 1996). Insulin-like growth factor-II binds to both the IGF-I receptor and the IGF-II receptor during the preimplantation stage (Werner et al., 1995). The transcription of IGF-I mRNA does not begin until after implantation, which occurs in the mouse after 8 to 9 days post concepttion (Hill, 1996). The two fundamentals roles for IGF during embryogenesis are; 1) regulation of birth size, and 2) control of muscle development (Hill, 1996). In a study using an IGF-I gene knock-out mouse model, *IGF-1(-/-),* neonate

birth weight was reduced by 60% compared with normal pups (Liu et al., 1993). Most newborn pups survived parturition, but died approximately 15 minutes to 6 hours later due to unknown reasons. This highlights the essential role of IGF in normal growth and development of the mouse. Only 16% of the homozygous mutants survived to adulthood (Liu et al. 1993). Liu e al. (1993) also investigated the effects of an IGF-I receptor knockout. These pups were born alive, but due to the inability to breathe died within minutes of birth. To investigate IGF-II, a double knock-out, *IGFr-1(-/-)/IGF-2(p-),* was used. The experiment had similar results to the IGF-I knockout, these pups were lighter at birth compared with wild type neonates (Liu et al. 1993). One difference observed knockout models was that the mice that survived to adulthood in the IGF-I group were infertile, whereas those from the *IGFr-1* group were fertile (De Chiara et al., 1990).

Insulin-like Growth Factor and Nutritional Status

Nutrition plays a key role in the growth and development of the fetus and the offspring postnatally. The developing fetus is reliant on the ability of the mother to transfer oxygenated blood and nutrients through the placenta for growth and development (Mullis et al., 2008). Postnatally, the level of nutrition of an animal is affected by its ability to find its own food, the quality of milk received from the mother, or the ability of the mother to provide additional food. Clemmons and Underwood (1991), observed that in human children that were both protein and calorie undernourished, serum concentrations of GH were increased and IGF-I were decreased. In ruminants,

there is a connection between nutritional status and IGF-I concentrations (McGuire et al., 1992). In growing cattle that have a severely restricted calorie/protein diet, circulating concentrations of IGF-I are reduced (McGuire et al., 1992). Sheep that were given a restricted diet during gestation produced lambs that were smaller and experienced poor postnatal growth (Vickers et al., 2001). Lambs were administered exogenous IGF-I which resulted in increased body weight (Vickers et al., 2001). Treatment with IGF-I negates some of the damaging effects of poor maternal nutrition by improving IGF-I receptor signaling, downstream signaling, and resistance to other metabolic hormones (Vickers et al., 2001). Dietary protein on its own is one of the greatest limiting factors in the diet of an animal and will result in the greatest decrease in circulating IGF-I (Cottam et al., 1992). When sheep were fed a well-balanced diet and treated with exogenous IGF-I for 8 weeks, circulating IGF-I increased by 60% compared with sheep on a control diet. The animals on a well-fed diet did not experience a change in body weight or food intake as expected (Cottam et al., 1992). Doubling IGF-I does not enhance the performance of animals with a normally functioning somatotropic axis functions normally (Cottam et al., 1992).

Insulin-like Growth Factor Binding Proteins

Less than 1% of circulating IGF is found unbound, in its free form, mediating its cellular actions (Collett-Solberg and Cohen, 1996). There are 6 IGFBP that transport IGF in circulation (Collett-Solberg and Cohen, 1996). The majority of IGF-I and IGF-II are found in a ternary complex that is composed of IGF-I, IGFBP-3, and an acid labile

subunit (ALS; Collett-Solberg and Cohen, 1996). The acid labile subunit is a glycoprotein produced by the liver, is found exclusively in the circulation, and is not capable of crossing the capillary barrier (Wetterau et al., 1999). Within cells, IGF is not attached to ALS, but it is still not in a free form, instead it is only bound to IGFBP (Wetterau et al., 1999).

The IGFBP have a highly conserved core-structure, with 50% homology across all IGFBP and 80% homology between species (Collett-Solberg and Cohen, 1996). The main difference between IGFBP is in the carboxy terminus; the termini differences define the affinity of IGFBP to IGF-I or IGF-II, as well as the difference in binding affinity to the extracellular matrix on the surface of a target cell (Hill, 1996). All IGFBP, except IGFBP-1 have a heparin binding domain which allows them to bind to sulfated glycosaminoglycans on the cell membrane (Hill, 1996). The structure of IGFBP-1 and IGFBP-2 include an integrin binding motif which allows them to bind to a fibronectin receptor (Hill, 1996). Some of the roles of the IGFBP include prolonging the half-life of IGF in circulation (Collett-Solberg and Cohen, 1996). Hypoglycemia, induced by free IGF, this aides in the passage of IGF from the intravascular space into the extravascular space, preventing an excess of free IGF from binding with the cellular receptors (Collett-Solberg and Cohen, 1996). This enhances the actions of IGF by creating a slow-release pool of IGF that can work independently in the cell (Collett-Solberg and Cohen, 1996). The main purposes of the IGFBP is to enhance or inhibit the actions of IGF, and is closely regulated through metabolic status (Umapathy et al., 2000).

Insulin-like Growth Factor Binding Protein-1

Insulin-like growth factor binding protein -1 was first isolated and characterized from amniotic fluid (Povoa et al., 1984). Povoa et al. (1984) reported that this binding protein was the most abundant of the binding proteins in amniotic fluid, and has an affinity for both IGF-I and IGF-II, but not to insulin. The gene for IGFBP-1 is 25,000 daltons and is located on the 7th chromosome in humans. In addition to its presence in amniotic fluid, IGFBP-1 is also produced by the liver, the decidua, and the kidneys, and found in the general circulation (Wetterau et al., 1999). The effects of IGFBP-1 have been reported as stimulatory and inhibitory, with its main role being the inhibition of IGF-I production (Collett-Solberg and Cohen, 1996). The concentrations of IGFBP-1 in circulation are dependent upon metabolic status (Collett-Solberg and Cohen, 1996). During times of fasting in children and/or adults, IGFBP-1 will increase to be greater than 100 ng/mL, but following a meal, serum concentration will decline to less than 10 ng/mL (Shimasaki and Ling, 1991). The predominate factors in regulating the concentrations of IGFBP-1 are insulin, corticosteroids, glucocorticoids, and glucagon (Shimasaki and Ling, 1991). Insulin and corticosteroids accomplish this through control of transcription of IGF in the hepatocyte (Wetterau et al., 1999). Increases and decreases in insulin concentrations result in changes in the synthesis of IGFBP-1, respectively, (Collett-Solberg and Cohen, 1996). Conversely, glucagon and glucocorticoids stimulate the production of IGFBP-1 and this increase in concentrations result in the repression of insulin (Collett-Solberg and Cohen, 1996). Insulin-like growth factor binding protein-1 can be secreted in a phosphorylated or non-phosphorylated state (Baxter, 2000). Depending upon its phosphorylated state, the affinity to IGF is

altered (Baxter, 2000). In humans, the affinity of the phosphorylated IGFBP-I is 6 times greater for IGF than the non-phosphorylated protein (Baxter, 2000). It is suggested that the proportion of phosphorylated versus nonphosphorylated is under hormonal control but is yet to be exactly defined (Baxter, 2000).

Insulin-like Growth Factor Binding Protein-2

Insulin-like growth factor binding protein -2 is a 31,000 dalton protein with the gene located on the long arm of chromosome 2 in humans (Wetterau et al., 1999). The entire gene has a total length of 32 kb; the largest portion of this gene is the first intron which is 27-kb (Shimasaki and Ling, 1991). Unlike IGFBP-1, IGFBP-2 is not phosphorylated or glycosylated, glycosylation is the attachment of a glycosyl donor to a hydroxyl group (Baxter, 2000). Concentrations of IGFBP-2 are dependent upon age, during infancy concentrations are increased and decline with age. A second increase occurs in cattle post-maturity (Cohen et al., 1992). In Hereford cattle, during the first 10 weeks of age, IGFBP-2 increased, which was followed by a decline in concentration to 24 weeks of age (Govoni et al., 2003). From 24 to 43 weeks of age, serum concentrations of IGFBP-2 remained constant (Govoni et al., 2003). Insulin-like growth factor binding protein-2 is highly expressed by neuroblastoma cell lines, leading to increased concentrations in cerebrospinal fluid (Chambery et al., 1998). In cases of extended fasting, the concentration of IGFBP-2 increases, which is even more pronounced in cases of severe protein restriction versus general caloric restriction (Wetterau et al., 1999).

Insulin-like Growth Factor Binding Protein-3

The most abundant of the IGFBP in animal and human serum is IGFBP-3 (Shimasaki and Ling, 1991). In humans, IGFBP-3 has a molecular weight between 40,000 and 44,000 daltons, and its gene is located on the 7^{th} chromosome along with the gene for IGFBP-1 (Katz et al., 1995). In rats, IGFBP-3 under non-reducing conditions has a molecular weight of either 56,000 daltons or 50,000 daltons (Shimasaki and Ling, 1991). Kumar et al. (2006) used ovine genomic DNA to show that the sheep IGFBP-3 gene was 654 bp in length. It had been demonstrated that cattle IGFBP-3 is 651 bp in length and buffalo 655 bp in length (Kumar et al., 2006). There are three separate sites on IGFBP-3 that have the ability to be glycosylated, Asn^{89}, Asn^{109}, and Asn^{172}. While the first two positions, Asn^{89} and Asn^{109}, are always glycosylated the third position Asn^{172} site is not always glycosylated resulting in the different possible molecular weight and changes to binding affinity (Baxter, 2000). Another cause of the difference in molecular weight is due to the phosphorylation of IGFBP-3, phosphorylation unlike glycosylation, does not affect binding affinity (Baxter, 2000).

The majority of IGFBP-3 is produced by hepatic tissue, but there are also a variety of other tissues that are capable of producing IGFBP-3 (Wetterau et al., 1999). For example, IGFBP-3 has been produced in porcine muscle satellite cells and porcine embryonic myogenic cells (Xi et al., 2006). Besides GH, there are various environmental and genetic factors that play a role in IGFBP-3 concentrations (Hennies and Sauerwein, 2003). The effects of IGFBP-3 are tissue dependent, and can either be inhibitory or stimulatory depending upon serum concentrations and IGF concentrations (Collett-Solberg and Cohen, 1996). Cell death can be modulated by IGFBP-3 through

either an IGF-independent pathway or dependent pathway (Kim et al., 2009). Although the exact mechanism on how IGFBP-3 induces apoptosis has yet to be fully described, it has been suggested that IGFBP-3 increases proapoptotic proteins (Kim et al., 2009). The effects of the increases in proapoptotic proteins are seen at the cell-surface receptor, the cytosol and the nucleus of the cell (Leibowitz et al., 2013).

Serum concentrations for IGFBP-3 in humans are between 1 to 4 µg/mL (Katz et al., 1995). Govoni et al. (2003) reported a gender effect in Hereford calves for average serum concentration between weeks 17 and 43 of age, males had a greater concentration of IGFBP-3, 93.1 ± 1.9 arbitrary units (AU), compared with 79.5 ± 1.9 AU in female calves. Additional regulators of IGFBP-3 concentrations are parathyroid hormone, estrogens, glucocorticoids, and nutrition (Katz et al., 1995). Rausch et al. (2002) showed that cattle fed 75% of the ration given to steers/heifers being fed ad libitum had a 13% reduction in serum IGFBP-3. Children who are malnourished, having minimal subcutaneous fat and 60% below normal body weight, had decreased concentrations of IGFBP-3, 1,635 ng/mL, compared with healthy children 3,726 ng/mL (Haspolat et al., 2007). This supports the link between nutrition and IGFBP-3 (Haspolat et al., 2007).

Insulin-like Growth Factor Binding Protein-4

Insulin-like growth factor binding protein-4 has an unglycosylated molecular weight of 24,000 daltons and a glycosylated weight of 29,000 daltons (Mazerbourg et al., 2004). The gene for IGFBP-4 resides on the 17th chromosome in humans (Wetterau

et al., 1999). The IGFBP-4 gene spans a total of 12 kb. It has four exons that will produce a single 2.6 kb mRNA (Kelley et al., 1996). There are a variety of cells that are capable of producing IGFBP-4, such as neuroblastomas, granulosa, fibroblasts, adrenal, thecal, testis, prostate, and bone cells (Shimasaki and Ling, 1991; Mohan et al., 1995; Armstrong et al., 1998). The greatest concentration of IGFBP-4 is found in bone, and the next greatest concentration is found in seminal plasma and blood serum (Katz et al., 1995). In pigs, IGFBP-4 is expressed in granulosa cells, in women it is expressed in granulosa and thecal cells, and while in sheep it is expressed only in thecal cells (Armstrong et al., 1998). One of the unique aspects of IGFBP-4 is that it inhibits the effects of IGF in all cell systems (Katz et al., 1995). Insulin-like growth factor binding protein-4 is the most widely produced binding protein in bone, therefore it has the greatest effect in decreasing proliferation of bone cells (Mohan et al., 1995). There is also a connection between circulating IGFBP-4 and age; humans between the ages of 61 and 87 years had 35% more IGFBP-4 compared with those between the ages of 23 to 40 years (Mohan et al., 1995). Increased GH, parathyroid hormone, and vitamin D3 control transcriptional regulation of IGFBP-4. Estradiol will inhibit transcription of IGFBP-4 in humans (Mazerbourg et al., 2004).

Insulin-like Growth Factor Binding Protein-5

Insulin-like growth factor binding protein-5 has an unglycosylated molecular weight of 29,000 daltons and a glycosylated weight of 29,000 to 32,000 daltons (Katz et al.,1995). In humans, the gene for IGFBP-5 is located on the second chromosome next

to the gene for IGFBP-2 (Wetterau et al., 1999). Similar to other IGFBP, IGFBP-5 concentrations change depending upon age; the greatest concentrations can be detected in fetal tissue, but decline following puberty (Collett-Solberg and Cohen, 1996). In rats administered exogenous IGFBP-5, cancellous bone formation and bone mass density increased in the spine and the femur (Hoeflich et al., 2008). In contrast to the trend reported in IGFBP-4, IGFBP-5 decreases in adults ages 61 to 71, compared with those between the ages of 23 to 40 years (Mohen et al., 1995). A link has been detected with ovarian function; in healthy follicles, the expression of IGFBP-5 is inhibited, whereas in degenerative follicles it is not inhibited (Kelley et al., 1995). Production of IGFBP-5 in the mammary gland has also been found to increase during involution of the mammary gland (Allan et al., 2004). Insulin-like growth factor binding protein-5 has a poorly understood role in apoptosis of mammary cells; however expression of IGFBP-5 is decreased there is a resulting delay in mammary gland involution in STAT3 knock-out mice (Allan et al., 2004). Insulin-like growth factor binding protein-5 plays a roll in the remolding of the mammary gland (Allan et al., 2004). In mammary epithelial cells, Sakamoto et al. (2007) reported that cells treated with GH had less IGFBP-5 expressed compared with control cells, demonstrating GH as an inhibitor of IGFBP-5.

Insulin-like Growth Factor Binding Protein-6

Insulin-like growth factor binding protein-6 is the only O-glycosylated IGFBP, with a molecular weight of approximately 34,000 daltons (Martin et al., 1994). The gene for

IGFBP-6 is located on the 12th chromosome, and has a distinct N-terminal amino acid sequence compared with the other IGFBP (Shimasaki and Ling, 1991; Neumann et al., 1998). The O-glycosylation makes IGFBP-6 unique in that it prevents proteolysis of this protein without affecting its binding affinity (Bach, 2005). The difference between IGFBP-6 and the other IGFBP is that the N-domain lacks two cysteine resides that are conserved in the other IGFBP (Chandrashekaran et al., 2007). One unique trait of IGFBP-6 is that it is the only IGFBP that binds IGF-II with a greater affinity than IGF-I; its main role via IGF-II is inhibition (Bach, 2005). The cells that express IGFBP-6 are fibroblasts, prostate cells, and ovarian cells; the expression is regulated mainly by IGF-II (Collett-Solberg and Cohen, 1996). When bound to IGF-II, IGFBP-6 has inhibitory actions, and has been reported to inhibit the growth of cancer cells that are IGF-II dependent (Chandrashekaran et al., 2007). In cell culture experiments, it was reported that increased IGFBP-6 decreased binding of IGF-II and caused suppression of IGF-II-dependent myoblast differention (Collett-Solberg and Cohen, 1996).

Fetal Programming

The concept of fetal programming, also termed developmental programming, is a relatively new concept in animal science, and was originally developed from human epidemiological studies (Barker, 1994). These studies showed that there is a critical time during early development that will result in permanent changes in development, growth and disease risk (Baker, 1995). For the fetus, the most common reason for changes to the expression of the fetal genome is changes to fetal nutrition and/or

endocrine status (Wu et al., 2006). This, in turn, results in adaptions in development, mainly organ and tissue structure, physiology, and metabolism to achieve ideal growth (Wu et al., 2006). These changes while in the uterus lead to the offspring being predisposed to endocrine or metabolic imbalance and/or cardiovascular disease later in life (Du et al., 2010). Fetal programming is important to the livestock industry because a fetus that fails to reach its growth potential negatively affects future production (Du et al., 2010). Fetal programming is most pronounced in young animals during their first parity, when carrying multiple fetuses, or when the animal experiences poor nutrition (Reynolds et al., 2010). In humans, 60 genes have been identified that are susceptible to imprinting (Myatt, 2006). Genomic imprinting is an phenomenon where the expression of certain genes are expressed based on parental origin (Wilkinson et al., 2007). Maternal genes that undergo imprinting suppress fetal growth, whereas the fraternal genes that undergo imprinting enhance fetal growth (Myatt, 2006). In humans, nutritional insults that are applied to the mother have the potential to cause diabetes or hypertension in adulthood in the progeny (Myatt, 2006). Fetal programming is not just a result in intrauterine growth retarded pregnancies but can also occur when the mother is suffering from other diseases, which commonly results in progeny that are small for gestational age or large for gestational age (Myatt, 2006).

Intrauterine Growth Retardation

Intrauterine growth retardation was defined by Wu et al. (2006) as the impaired growth and development of the mammalian embryo/fetus or its organs during

pregnancy. It can be caused by a variety of factors both intrinsic and extrinsic. Over the past 50 years there have been significant advances in management techniques and intensive research into the field; however, IUGR is still a problem for the animal industry (Wu et al., 2006). Genetics is the main determinant of fetal development and growth, with the genetic code is vulnerable to changes brought on by the uterine environment (Holemans et al., 1998). Several effects linked to IUGR are reduced postnatal growth, reduced neonatal survival, reduced feed efficiency, negative impacts on meat quality, and negative impacts on body composition (Wallace et al., 1997; Peel et al., 2012). The impacts of IUGR is caused by several factors, including uterine capacity, inadequate amniotic or allantoic fluid, maternal nutrition, maternal intestinal malabsorption, ingestion of a toxic substance, placental abnormalities, maternal age, poor management, infection, environmental temperature, stress, and disturbances in both the maternal and fetal homeostatic mechanisms (Wallace et al., 1997; Luther et al., 2007). An interesting concept about IUGR was defined by Schroder (2003), who described the uterine-placental unit as a “Russian nesting doll motif”. The Russian nesting doll is described as an object within a similar object. What is important regarding this concept is that each of these units fit within each other and is affected from the upstream unit. The fetus is affected by the uterine environment, and the uterine environment is affected by maternal homeostasis (Schroder et al., 2003). When investigating IUGR it is important to take into consideration that the different components interact with each other and that they are all connected with the growth of the fetus.

Intrauterine growth retardation can be caused by intrinsic or extrinsic natural circumstances or other factors. Natural IUGR, was defined by Jensen et al. (1999) as

reduced substrate delivery to the fetus or impaired placental efficiency. One natural/intrinsic cause of IUGR is uterine capacity. Uterine capacity induces IUGR as reported in studies investigating the effects of uterus size and capacity in pig pregnancies (Foxcroft et al., 2006). Depending upon the stage of fetal growth, the uterus can exert different effects upon the growing fetus. Not until day 25 of gestation will survival of the offspring begin to be affected (Foxcroft et al., 2006). Moreover, its not until day 30 to 40 that the uterine environment will permanently affect the fetus (Foxcroft et al., 2006). Overcrowding in multi-fetal pregnancies is one of the most common causes of natural IUGR in livestock animals (Chen et al., 2011). For example, pigs have the greatest percentage of IUGR pregnancies (Chen et al., 2011). Due to placental insufficiency these IUGR piglets are 0.3 kg lighter at birth than normal (Chen et al., 2011). Before 35 days of gestation pig fetuses are equally distributed within the uterine horns, it is not until after this time point that they begin to crowd each other and compete for space (Wu et al., 2006). In the sheep carrying twins, each lamb will be on average 78% of the body weight of a singleton lamb, whereas in triplet pregnancies, the average fetal weight per lamb was 62% compared with a singleton (Gootwine, 2005). Even when the ewe is receiving adequate nutrition, in multi-fetal pregnancies, which account for 38 to 52% of all sheep pregnancies (USDA, 2003), fetal growth is impaired (Gootwine, 2005). Uterine capacity can be both a natural or experimental cause of IUGR. Intrauterine growth retardation can be experimentally induced by transferring an embryo from a large uterine capacity mother into a mother who has a small uterine capacity (Sharma et al., 2012). Sharma et al. (2012) compared conceptuses from two different-sized sheep breeds developed in utero. Suffolk sheep, known for large body

size and large uterus and Cheviot sheep, known for small body size and small uterus were used (Sharma et al., 2012). Embryos were implanted either within a breed or between breeds, with the aim to determine the effect of uterine size on growth and development (Sharma et al., 2012). Sharma et al. (2012) reported that the largest lambs produced lambs that were born to the natural Suffolk x Suffolk. With Suffolk embryos gestated in the smaller Cheviot uterus were lighter at birth, showing the impact of uterine size on fetal size. No difference was seen in Cheviot in Cheviot or Cheviot in Suffolk in regards to birth weight (Sharma et al., 2012). This is a clear example of how the fetus adapts its growth and development to compensate for extrinsic factors.

Nutritional Status during Gestation and IUGR

Maternal nutrition is one extrinsic factor that impacts maternal homeostasis and their fetal development. Overfeeding and/or underfeeding constitute poor maternal nutrition and has negative implications on the offspring. Undernutrition can be the result of poor management practices, animals being raised on harsh range conditions, or animals being housed in a group environment where competition for food may lead to some receiving less than others (Wu et al., 2006; Lekatz et al., 2011). Pigs, for example, are commonly group-housed, and need to compete with others for food, whereas large livestock such as horses and cattle are grazed on rangeland, which might be over-grazed or poorly managed (Wu et al., 2006). Vonnahme et al. (2008) reported that sheep fed 50% of NRC requirements carried fetuses with a lighter body weight than lambs carried by ewes fed 100% NRC requirements. It was reported that

lambs from the restricted-fed ewes had a decreased crown rump length compared with control-fed ewes lambs (Vonnahme et al., 2006). In pigs, those that were considered light weight at birth grew slower and were smaller than pigs born at a medium or heavy body weight (Rehfeldt et al., 2007). In cattle, very light body weight before calving resulted in a reduction in calf birth weight (Bellows et al., 1971). Overnutrition also negatively impacts livestock production. For example in sheep, overfeeding is common practice pre-breeding, this process is known as flushing and increases the ovulation rate of the dam. In addition to overfeeding before pregnancy, overfeeding during gestation can negatively impact the fetus (Wu et al., 2006). In pigs fed a high energy and/or high-protein diet, there is an increased risk of embryo mortality (Wu et al., 2006). In horses that were overconditioned in late pregnancy, the mares experienced increased fetal loss and decreased fetal growth rates (Pugh, 1993). An additional example is the overfeeding dairy cattle, overfeeding does not decrease dystocia or increase milk production, instead it results in an increased rate of ketosis, cystic ovaries, loss of fertility, and increased risk of metritis (Ferguson, 2005). In conclusion overfeeding during gestation can result in increased fetal loss, decreased fertility, decreased fetal growth rates, and lacks ample benefits in the production setting.

Maternal Nutrition and the Somatotropic Axis

The somatotropic axis hormones of both the fetus and mother are under a high degree of regulation in mid- to late-gestation by maternal nutritional status (Oliver et al., 1996). Nutrient restriction of the ewe from day 28 of gestation until termination of

gestation, resulted in fetuses at day 135 of gestation with a significantly reduced number of somatotrope cells compared with an increase in somatotrope cellular area within the pituitary gland (Lutz et al., 2006). The decrease in number of somatotrope cells challenges the increase in GH concentration in the lambs (Lutz et al., 2006). The reason for the disconnect between number of somatotrope cells and concentration of GH was postulated to be due to new somatotrope cells being undetectable by immunocytochemistry since these cells have exhausted their stored GH (Lutz et al., 2006).

Embolism is the process of creating an obstruction in a vein or artery to slow blood flow and can be utilized experimentally as a method of inducing IUGR in the fetus (Jensen et al., 1999). Jensen et al. (1999) used microspheres injected into the uterine arteries to create an embolism that slowed uterine blood flow, limited blood gases and nutrients from reaching the fetus. The purpose of the embolized artery is to induce reduced fetal growth and allow for the ability to track the associated changes in development (Eremia et al., 2007). Romney ewes were assigned to be either embolized or embolized and given exogenous IGF-I treatment, had catheters inserted into the fetal and maternal veins to collect blood samples to track metabolic changes (Eremia et al., 2007). The IGF-I treatment group was an attempt to counteract the effects of the embolism by returning the fetus to normal growth (Eremia et al., 2007). To track changes caused by embolism-induced IUGR, one treatment group was injected with saline (Eremia et al., 2007). Overall, the induced IUGR/saline treatment group reported a decreased fetal growth rate of 20 to 30% compared with controls (Eremia et al., 2007). In treated groups, intravenous IGF-I supplementation resulted in a fetal chest

girth circumference that was greater than lambs who were administered saline, fetal weight was lighter for IGF-I treatment compared with controls and heavier than the saline treatment group (Eremia et al., 2007). In conclusion, this study reported that when the somatotropic axis is not functioning correctly due the decrease in nutrient transfer through the uterine artery, but treatment with exogenous IGF-I can correct the imbalance and improve growth and development (Eremia et al., 2007).

Maternal Nutrition and Adipose Tissue

There have been numerous studies (Flint et al., 2003; Haemmerle et al., 2006; Berryman et al., 2011; Long et al., 2012) that suggest that maternal obesity or maternal disease state can program the fetus during gestation. Long et al. (2012) showed these adaptions occurred in adipose tissue related to adipogenesis or lipolysis. A change in the fetal fat mass was linked to the decreased nutrient availability due to alterations in maternal nutritional status (Long et al., 2012). To confirm these changes in fetal adiposity, Long et al. (2012) designed a study in which ewes were overfed to the point that at midgestation fetal carcass weights (day 65 to 75 of gestation) were 30% heavier than controls. At day 135 of gestation, the total fetal weight of lambs born to obese ewes and control ewes was similar, but when just the carcass weight was compared the lambs born to obese ewes (3.56 kg) were significantly lighter compared with the control-born lambs (4.12 kg; Long et al., 2012). When the morphology of adipose cells was analyzed at day 135 of gestation, it was reported that the cells from pericardial depots from the lambs of obese ewes had a larger diameter than depots in lambs from the

control ewes (Long et al., 2012). This was also reported for cells from the perirenal fat depot (Long et al., 2012). Backfat in lambs from obese ewes was twice as thick as the backfat from control lambs (Long et al., 2012). The cells of lambs from obese ewes that were from the perirenal depot also had a greater concentration of fatty acids within the cell compared with those from control ewes (Long et al., 2012).

Long et al. (2011) used sheep as a model to evaluate leptin, a hormone produced by adipocytes, and how it changes with maternal obesity. Leptin is essential for the maintenance of postnatal body weight and development of the appetite control center (Monteleone and Maj, 2013). During postnatal development there is an increase in circulating leptin within days of parturition; in rats this is observed on postnatal days 8 to 21 (Kirk et al., 2009). This peak of leptin has a role in programming the appetite center of the brain before concentrations return to a basal level (Kirk et al., 2009). Obese ewes were fed 150% of NRC requirements for the duration of gestation, blood samples were taken following parturition in the lambs to quantify concentrations of leptin (Long et al., 2011). Although birth weights were similar between treatment groups, lambs from control ewes had an increase in leptin starting at day 5 to 9 postnatally. The leptin peak did not occur in lambs from obese ewes compared with control ewes (Long et al., 2011). This supports the previous study done by Long et al. (2010) that showed the lambs from overfed ewes consumed 10% more than control lambs, and had a tendency to be heavier than lambs from control ewes with a greater percentage of fat (20.8% vs 16.5%; Long et al., 2010).

Maternal Nutrition and Muscle

The critical period in which myogenesis occurs in pigs is between day 25 and 90 of gestation (Foxcroft et al., 2006). Dwyer et al. (1994) showed that maternal nutritional status had its greatest effects on the fetus between days 25 to 50 of gestation. Between days 25 and 90 of gestation the primary and secondary muscle fibers are experiencing their greatest growth and most vulnerable to manipulation (Dwyer et al., 1994). Using pigs, it was shown that decreased maternal nutrition during gestation resulted in reduced numbers of secondary muscle fibers and a reduction in birth weight (Handel and Stickland 1987; Dwyer et al., 1994). Piglets experiencing IUGR will be more likely to have a reduction in muscle mass and meat quality at the time of slaughter (Rehfeldt et al., 2008). When piglets are designated into one of three birth weight groups (low weight, middle weight, and high weight), at the time of slaughter the animals from the high birth weight group were superior to those from the low birth weight group (Rehfeldt et al., 2008). Superior was defined by an increase in lean muscle and decreased fat, the low birth weight pigs were clearly inferior having less lean muscle mass and more fat than pigs from the high birth and medium weight groups (Rehfeldt et al., 2008). Interestingly, though the piglets from each of the three birth weight groups had similar backfat thickness, the additional fat reported in the low birth weight pigs was in perirenal fat (Rehfeldt et al., 2008). The differences in carcass quality shows that birth weight might have a greater effect on muscle development than adipose tissue development and that differences in adipose tissue development will be depot specific (Rehfeldt et al., 2008). Low birth weight lambs had a 40% reduction in semitendinosus muscle weight compared with high birth weight lambs (Greenwood et al., 2000). Differences in

how lambs were reared, (eg., for slow rate of body weight gain or fast gain rate of body weight gain), muscle weight and carcass weight were affected (Greenwood et al., 2000). Low birth weight lambs had a slower daily body weight gain compared with high birth weight lambs and a reduced muscle weight (Greenwood et al., 2000). Greenwood et al. (2000) concluded that nutrition during early postnatal life the growth potential of sheep and muscle growth will be altered by nutrition. In conclusion, it can be postulated that nutrition affects late prenatal growth similarly as it does during early postnatal growth (Greenwood et al., 2000).

Overfeeding, another form of poor maternal nutrition, also affects postnatal growth negatively. When pigs were overfed from conception to day 50 of gestation, the offspring were fatter at birth with altered muscle fiber composition (Bee, 2004). The piglets born to overfed sows grew slower, had a reduced percentage of muscle, and heavier fat mass compared with piglets born to restricted fed sows (Bee, 2004). It was also shown that rats that were overfed during gestation had a 25 % reduction in muscle cross-sectional area and additionally had 20% fewer muscle fibers compared with control-fed mothers (Du et al., 2010). Ewes fed 150% of NRC requirements produced lambs with reduced expression of MyoD, myogenin, and the protein desmin, signifying downregulation of myogensis (Tong et al., 2009). Tong et al. (2009), also reported that lambs born to obese ewes had a reduction in primary muscle fiber size compared with control born lambs. Typically, the downregulation of myogensis is associated with the increase in adipogensis and fibrogensis due to divarication of the mesenchymal stem cells (Tong et al., 2009). In conclusion, an obese mother during gestation results in

offspring with decreased muscle fibers and muscle fiber size (Tong et al., 2009; Du et al., 2010).

Summary

Poor maternal nutrition has numerous effects on the growth and development of the offspring. When receiving sub-optimal nutrition during gestation, the offspring are lighter at birth than those born to mothers receiving a control diet (Bellows et al., 1971; Vonnahme et al., 2008; Chen et al., 2011). Offspring born to nutrient restricted mothers also have decreased muscle mass (Handel and Stickland, 1987; Dwyer et al., 1994; Rehfeldt et al., 2007). The effect of poor maternal nutrition on the offspring in regard to the somatotropic axis is poorly understood. Using the technique of embolizing the main uterine artery thus creating a disruption in nutrient flow to the fetus, treatment with IGF-I have been reported to increase fetal growth (Eremia et al., 2007). Growth hormone has been reported to be increased in growing animals that have undergone prolonged fasting (Breier, 1999). Insulin-like growth factor binding protein-3 is decreased in cattle that are only being fed 75% of NRC requirements, whereas IGFBP-2 was reported to increase in cases of extended fasting (Wetterau et al., 1999; Rausch et al., 2002). These data support the concept that nutrient restriction to the fetus decreased IGF-I, decreased IGFBP-3, increased GH, and increased IGFBP-2 concentrations and decreased growth and development. It would be beneficial to examine hormones of the somatotropic axis in a maternal restricted nutrient model. Quantifying these hormone concentrations in relation to body parameters will make it possible to understand the endocrine system response and adaption with maternal nutrient restriction to optimize

growth and development of the offspring. Understanding these changes will allow producers the ability to optimize the growth and development of livestock.

Similarly overfeeding an animal during gestation impacts growth and development of the offspring. Livestock that were fed in excess of NRC requirements during gestation had increased fetal mortality and decreased fetal growth (Pugh et al., 1993; Wu et al., 2006). Obese mothers have been reported to have offspring with increased fat depots, and increased adipose cell size (Long et al., 2010, 2011). Muscle has also been reported to be affected in cases of maternal overnutrition. Postnatally offspring that were fatter at birth grew slower and had less lean muscle at the time of slaughter (Bree, 2004). Muscle cross sectional area will be reduced in offspring with overnutrition of the dam, with muscle fibers specifically being reduced (Du et al., 2010). The impact of poor maternal nutrition on the hormones of the somatotropic axis is poorly reflected in current literature and requires further study.

Objectives

It has been hypothesized that the effects of maternal nutrition have lasting postnatal effects on growth and development (Wu et al., 2006; Vonnahme et al., 2010; Howie et al., 2012; Hoffman et al., 2012; Neupane et al., 2012). Poor maternal nutrition, be it overnutrition or undernutrition, can result in IUGR, and has been linked to lasting effects on multiple organs, tissues, and endocrine systems (Foxcroft et al., 2006; Wu et al., 2006; Chen et al., 2011). The somatotropic axis is affected in lambs born to ewes that experienced poor maternal nutrition and IUGR. We hypothesized that overfeeding or underfeeding ewes during gestation would alter the growth response of lambs and the somatotropic axis. Therefore, the objectives of this study were:

1. To determine the effects of ewe undernutrition and overnutrition during gestation on growth variables of lambs (body weight, average daily gain, heart girth circumference, and crown rump length). These variables are indicators of postnatal growth, and will be measured in lambs from poorly nourished ewes from birth until three months of age.
2. To determine the effects of maternal nutritional status on circulating concentrations of GH, IGF-I, IGFBP-3 and IGFBP-2 in lambs. These hormones will be quantified to monitor changes in the endocrine response in lambs from birth to three months of age.
3. To determine the effects of maternal nutrition on carcass quantity (backfat, loin eye area) heart weight and liver weight, carcasses will be quantified in lambs from birth and three of age. These data will be to monitor differences in tissue and organ system growth and development.

Materials and Methods

Ewes

Dorset (n=21) and Shropshire (n=3) multiparous (n=18) and primiparous (n=6) ewes between 2 and 7 years of age were used for this study. All animals were born and bred at the University of Connecticut Sheep Barn. Before the study, animals were housed unrestrained, in pens with an attached outdoor exercise yard. All ewes were ultra-sounded to determine number of fetuses and approximate age of gestation. Ewes that were confirmed pregnant with twins and near 87 days of gestation were used in the study. Ewes were randomly assigned to one of three diet based treatment groups, Overfed (n=8) target of 140% of National Research Council (NRC) requirements, Control (n=8) target of 100% of NRC requirements, and Restricted (n=8) target of 60% of NRC requirements. The actual diet that was consumed by each treatment group was 120% for Overfed ewes, 100% for Control ewes, and 60% for Restricted ewes (Table 1). Ewes on the study began a 9-day transition period to their assigned treatment diet, and were consuming the experimental diet at approximately 114 days ± 10 of gestation. Breed was balanced across treatments, one Shropshire, two primiparous Dorsets and 5 multiparous Dorsets were included in each treatment group. Ewes remained on study until parturition.

One control ewe died post parturition from a uterine prolapse. The data collected (blood samples and weights) from the euthanized ewe before parturition were included the analysis.

Ewes were housed in individual pens to ensure each consumed only their experimental diet. Pens were bedded with straw and cleaned weekly. Ewes were given water ab libitum.

Treatments

Diets were fed to ewes once a day at 0800 h. At this time, ewes were given hay and cracked corn, based on treatment assignment and body weight (Table 2). Hay was presented in individual hay bags, and cracked corn fed in individual troughs. The following morning hay bags were removed from each animal and any remaining hay was weighed, discarded, and the amount recorded. No corn remained the following day. Ewes were given fresh hay each morning. All animals were given a mineral supplement and ad libitum access to fresh water in a heated bucket. One ewe lost excessive body weight (weighed less than 20% of weight-matched controls and was switched to control diet).

Ewe Body Weight, Body Condition Score, and Sample Collection

Ewes were weighed weekly, and body condition score (BCS) was determined. At least two BCS scores were obtained each sampling day, by trained individuals, and their scores averaged.

Every week, blood samples (20 mL) collected via jugular puncture. The 20 mL of blood was equally distributed into three tubes (no anticoagulant, heparin, and EDTA). Tubes for serum were stored at room temperature for 6 hours, then overnight at 4℃.

Table 1: Hay and Corn: Mean Total Digestible Nutrients (TDN) and Crude Protein (CP)

Treatment	Hay TDN (kg)	Hay CP (kg)	Corn TDN (kg)	Corn CP (kg)	Total TDN (kg)	Total CP (kg)	TDN vs Con (%)	CP vs Con (%)
Restricted	0.704	0.105	0.198	0.022	0.902	0.127	60	36
Control	1.25	0.308	0.351	0.038	1.609	0.346	-	-
Overfed	1.292	0.319	0.737	0.132	2.029	0.451	126	130

Table 1: Nutrient content of the corn and hay given in the restricted, control and overfed diets. Control is 100% NRC requirements, TDN vs. Con is 60% of NRC for restricted treatment group, overfed is 120% NRC.

Table 2: Average Hay and Corn Consumption for Treatment Groups across Entire Study

Treatment	Hay (kg)	Corn (kg)
Restricted	1.2	0.2
Control	2.1	0.3
Overfed	2.2	1.3

Table 2: Restricted group was fed first cutting hay. Control and overfed group were fed second cutting hay.

The following morning, the serum tubes were centrifuged at 3,000 x *g* at 4℃ for 30 minutes. Serum was harvested, placed in a polypropylene collection tube and frozen at -20℃ until analysis. The remaining tubes were inverted 8 to 10 times and placed on ice for 1 to 2 hours, and then centrifuged (3,000 x *g*, 30 min at 4℃). Plasma was harvested, placed in a polypropylene collection tubes and frozen at -20℃ until analysis.

Lambing Procedure

Ewes were moved to a lambing pen, at or near parturition. Following delivery, lambs were maintained under heat lamps and allowed to nurse the ewe for 24 hours. If it was determined by the observing student that insufficient colostrum was consumed, lambs were given commercial colostrum per manufacturer's instructions (Lamb's Choice Total Colostrum, The Saskatoon Colostrum Co; Saskatoon, Canada) via gastro-intestinal tube. Within 24 hours of birth, lambs body weight, crown-rump length, and heart girth circumference were measured. The lambs that would be included in the study were selected based on gender, males chosen over females, and heavier body weight. Blood (10 mL) was collected from each lamb designated to remain on the study. At 24 hours postpartum, ewes were returned to the flock with their remaining lamb(s).

Based on ultrasound data, each ewe was carrying twins; however from the 24 ewes there were 7 sets of triplets, 14 sets of twins, and 3 singletons. One lamb from each set triplets or twins was selected, and all singletons were selected for the study (n=24).

Lambs

Nine lambs (n=3/treatment) were slaughtered within 24 hours of birth. Fifteen lambs (n=5 Overfed, n=5 Control, and n=5 Restricted) were removed from the ewes 24 hours after birth and placed on a bottle fed milk replacer diet.

Two lambs died during this portion of the experiment; one due to enterotoxemia at one month of age and one lamb at two months of age, because of urinary calculi. Both lambs were born from ewes in the Overfed treatment group, reducing the total number of lambs in that treatment group to 3. Data from both lambs at birth were included in data analysis.

Bottle Fed Lamb Procedure

Fifteen lambs (n=5 Overfed; n=5 Control; and n=5 Restricted) were placed in group pens (3 to 4/pen) 24 hours after birth. Lambs remained in their pens for 3 months. Lambs were bottle fed milk replacer (Ultra Fresh Lamb Milk Replacer, Land O'Lakes Milk Product Company, Shoreview, MN) at 1.7% of body weight. For the first two weeks, lambs were fed (average = 812 ± 10 mL) milk replacer every three hours. From 3 to 4 weeks of age, animals were fed (average = 980 ± 12 mL) every 6 hours. From 5 to 8 weeks the milk was diluted, next a feeding per week was deducted until weaning was completed. On average, the lambs consumed 780 ± 10 mL per feeding during dilution, 586 ± 11 mL per feeding during 3 feedings, 390 ± 13 mL per feeding during 2 feedings, and 195 ± 9 mL when fed once per day. All lambs were weaned by approximately d 60 of age and all animals were provided creep feed ad libitum (Lamb BT, Blue Seal Feeds,

Litchfeild, CT), and second cutting hay to achieve a body weight gain of 0.30 kg/d. Creep feed and hay was first introduced to the lambs at the beginning of the weaning process.

Lambs were vaccinated for *Clostridium perfringins type C and D, and clostridium tetani* (Bar-Vac CD/T, Boehringer Ingelheim, St. Louis, MO) and sore mouth at 24 h and 14 d of age.

Lamb Body Weights and Sample Collection

Lambs were weighed three times per week. Crown rump length was measured weekly. Blood samples (10 mL) were collected via jugular venipuncture once per week for two weeks postpartum and then every two weeks until three months of age. Blood samples were handled as described above.

Necropsies

The nine lambs designated for 24-h slaughter were transported (0.2 km) to necropsy, a blood sample (10 mL), crown-rump length, and heart girth measurements were collected. The blood was handled as described above. The animals were euthanized with a commercial euthanasia solution (10 mL; Beuthanasia Special-D, Merck Animal Health, Summit, NJ) at 0.5 mg/kg. Once respiration and heart-beat ceased, the animal was exsanguinated.

At necropsy, tissue samples were collected from the heart, liver, kidney, longissimus dorsi muscle, quadricep muscle and perirenal brown adipose. Samples were snap-frozen in liquid nitrogen to prevent RNA degradation. Heart and liver weights were collected. Loin eye area and back-fat thickness were measured at the 12^{th} and 13^{th} rib. Backfat was not quantifiable for the 24-h slaughter group. Backfat measurements were quantifiable for the three month slaughter group and were analyzed as a percentage of body weight.

The 13 lambs designated for the three month slaughter groups were handled the same as above. Additionally, perirenal white adipose tissue was collected at three months of age.

Growth Hormone Concentration

Serum was used to determine circulating concentrations GH by radioimmunoassay (RIA) according to Kazmer et al. (1992). The standard curve was prepared through a serial dilution of a GH standard containing 40 ng/mL using assay buffer. Sample tubes were prepared in duplicates. Approximately 20,000 cpm of I^{125} GH were added per tube. Primary antibody (NIDDK, anti-oGH) was used at a dilution of 1:20,000. The bound radioactivity of each tube was determined using a gamma counter (ISO Data 20/20, Ramsey, Minnesota). One assay was run with triplicates of each sample.

Insulin-like Growth Factor-I Concentration

Serum was used to determine circulating concentrations IGF-I by RIA according to Govoni et al. (2002). Insulin-like growth factor-1 was dissociated from IGFBP and ALS through extraction using glycylglycine hydrochloric acid (Govoni et al., 2002). The standard curve was prepared through a serial dilution of an IGF-I standard containing 1,280 ng/mL using assay buffer. Sample tubes were prepared in triplicate. The amount of I^{125}-IGF-I (Perkin Elmer, Ma) added was calculated to 10,000 cpm per tube. Primary antibody (NIDDK, rabbit-anti-human-IGF-I) was used at a dilution of 1:150,000. The bound radioactivity of each tube was determined using a gamma counter (ISO Data 20/20). All samples were run in duplicate within one assay.

Insulin-like Growth Factor Binding Protein Concentration

IGFBP-3 and -2 concentrations were determined by the protocol described by Freake et al. (2001) and Govoni et al. (2002). Proteins were separated using 0.4% SDS-Polyacrylamide gel electrophoresis, separation was based on size. The gels were made in a Mini-protean II multi-casting chamber (BioRad, Hercules CA) at 30% acrylamide. Two microliters of serum were used and samples were loaded in duplicate with two control lanes. Non-glycosyated human IGFBP-3 (Diagnostic System Laboratories Inc, Webster TX) and bovine IGFBP-2 and -3 were used as standards. Electrophoresis was done at 100 V until the dye front passed into the stacking gel (~30 min), then run at 150V until dye front passed off the gel entirely (~90 min; power supply Model 250 Life Technologies, Gaithersburg, MD). Next, proteins were transferred via electro-blotting, at 45 V for 90 minutes, onto a nitrocellulose membrane (BioRad, Hercules CA**).** Membranes were stored at -20℃ until ligand binding.

Membranes were washed with 10 to 15 mL NP40/Igepal and 1% Tween, than incubated with I^{125}-IGF (300,000 cpm) for 15 hours. The membranes were then washed with additional steps of 0.1% Tween and TBS to block the membranes. Membranes were dried, wrapped in plastic wrap and set on a Phosphor Film in an X-Ray cassette to incubate in the dark for 18 hours. Films were scanned using a cyclone phosphor imager (Packard Instruments, Waltham, MA). Images were analyzed using OptiQuant to determine the amount of binding proteins in Digital Light Units (DLU) as a percent of control.

Additional Hormone and Metabolite Analysis

Triiodothyronine (T3; Calbiotech, Spring Valley, CA), thyroxine (T4; Calbiotech, Spring Valley, CA), were quantified in samples at birth and 3 mo using commercially available ELISA kits according to the manufacturer's protocol. Total cholesterol (TC) and triglycerides (TG) were quantified in samples at birth and 3 mo according to Rasmussen et al. (2009).

Statistical Analysis

Data were analyzed using Proc-Mixed model (SAS Inst. Inc., Cary, NC) . To analyze age-related changes over time and treatment differences for body weight, crown-rump length, GH, IGF-I, IGFBP-3, and IGFBP-2 repeated measures using the mixed-model ANOVA procedure of SAS was used. Treatment differences for loin eye area, backfat, heart weight, liver weight, heart girth circumference, leptin, glucose, T3,

and T4 were analyzed using the mixed-model of SAS. Significance was determined at $P \leq 0.05$ and tendencies were determined at $P \leq 0.10$ and P □ *0.05.*

Results

Body Weight and Average Daily Gain

At birth (n=24), lambs born to restricted-fed ewes (res-lamb) weighed 4.15 ± 0.33 kg (*P=0.01* vs. control). Lambs born to control-fed ewes (con-lamb) were similar (5.33 ± 0.33 kg) to lambs born to overfed ewes (over-lamb; 5.12 ± 0.33 kg; *P=0.66;* Figure 1). At birth, res-lambs weighed 25% (1.18 kg) less than the con-lambs (*P=0.01)* and 21% (0.97 kg) less than over-lambs (*P=0.04)*. Over the course of the experiment res-lambs weighed on average16% less than con-lambs (*P=0.01)*.

Between weeks 0 to 6 (Figure 1) control born lambs and lambs born to overfed ewes were similar (*P□0.20);* however, at week 7 and 8 con-lambs were 3.33 kg and 3.42 kg heavier, respectively, than over-lambs (*P=0.01;* Figure 1). At week 9 and 10 con-lambs were 2.56 kg and 2.37 kg heavier than over-lambs (*P=0.06*; *P=0.08*), respectively. After week 11, body weights of over-lambs were not different from control (30.33 kg vs 30.74 kg; *P=0.77)*.

At three months of age the res-lambs, con-lambs and over-lambs averaged 31.29 ± 1.35 kg, 35.21 ± 1.35 kg, and 33.59 ± 1.75 kg body weight, respectively (*P=0.17;* Figure 1). Res-lambs were 12% (3.9 kg) lighter than con-lambs (*P=0.06*) but there was no difference between con-lambs and over-lambs, (*P=0.48)*, and res-lambs and over-lambs (*P=0.32).*

Overall lambs gained an average of 28.50 ± 1.37 kg over the course of the experiment. Res-lambs gained 29.88 kg, con-lambs gained 27.14 kg, and over-labs gained 28.47 kg (Figure 1). Average daily gain for the lambs was 0.32 kg/day, with res-lambs gaining 0.29 kg/day, con-lambs gaining 0.33 kg/day, and over-lambs gaining 0.32 kg/day (*P=0.16)*.

Heart Girth Circumference

Heart girth circumference was measured at birth and three months of age (Figure 2). At birth, heart girth circumference average 36.90 ± 0.831 cm, 40.79 ± 0.831 cm, and 40.40 ± 0.831 cm for res-lambs, con-lambs, and over-lambs*,* respectively. Con-lambs heart girth circumference was 10% greater (3.89 cm) than res-lambs (*P□0.01*). Over-lambs had 9% (3.5 cm) greater heart girth circumference than res-lambs (*P□0.01;* Figure 2*).*

At three months of age, heart girth circumference was 70.04 ± 3.28 cm, 79.43 ± 3.28 cm, and 72.81 ± 4.24 cm for res-lambs, con-lambs, and over-lambs (*P=0.17;* Figure 2*)*, respectively. Res-lambs had 12.5% (9.42 cm) less heart girth circumference compared with con-lambs (*P=0.07*). Con-lambs increased 5.5 cm compared with res-lambs, and increased 6.22 cm compared with over-lambs.

Figure 1: Body Weights for all Lambs, from Birth to Three Months of Age

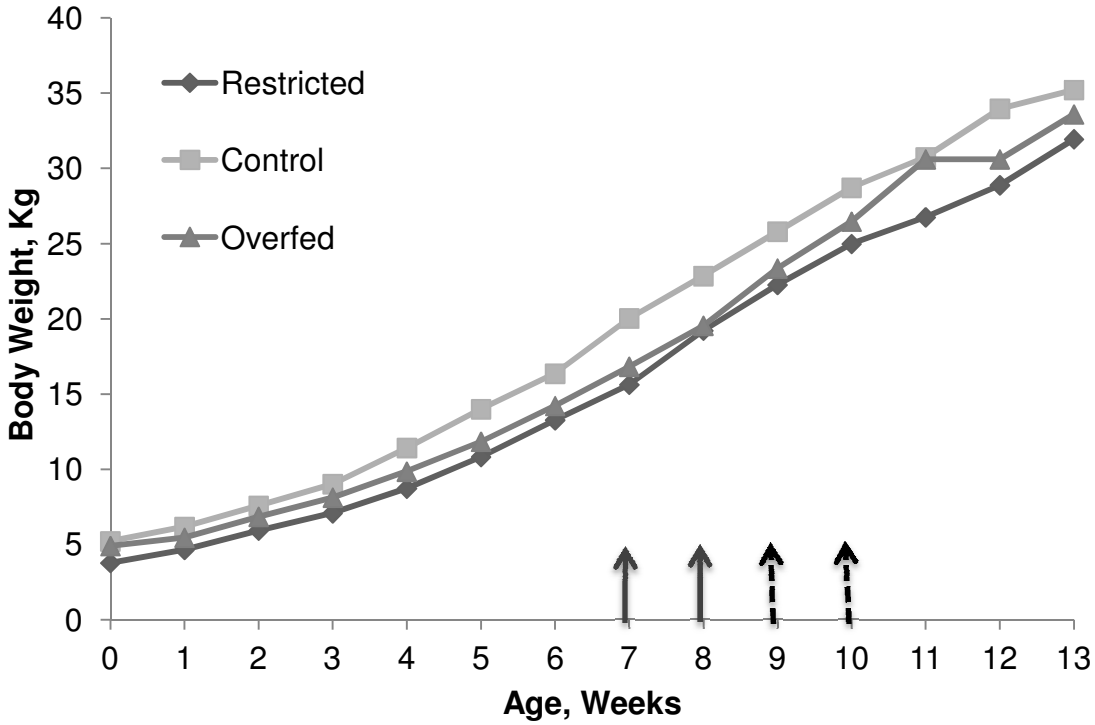

Figure 1: Body weights of lambs born to restricted-fed, control-fed, and overfed ewes from birth to three months of age (n=13). Body weights were reduced in res-lambs compared with con-lambs (P=0.01). At weeks 7 and 8, (bold arrows) over-lambs were 3.69 kg and 3.37 kg lighter than con-lambs (P=0.01), respectively. Body weights of over-fed lambs were 2.56 kg and 2.37 kg lighter at week 9 and 10 (dashed arrows; P=0.06; P=0.08), respectively. SEM = 0.63

Figure 2: Heart Girth Circumference, at Birth and Three Months of Age

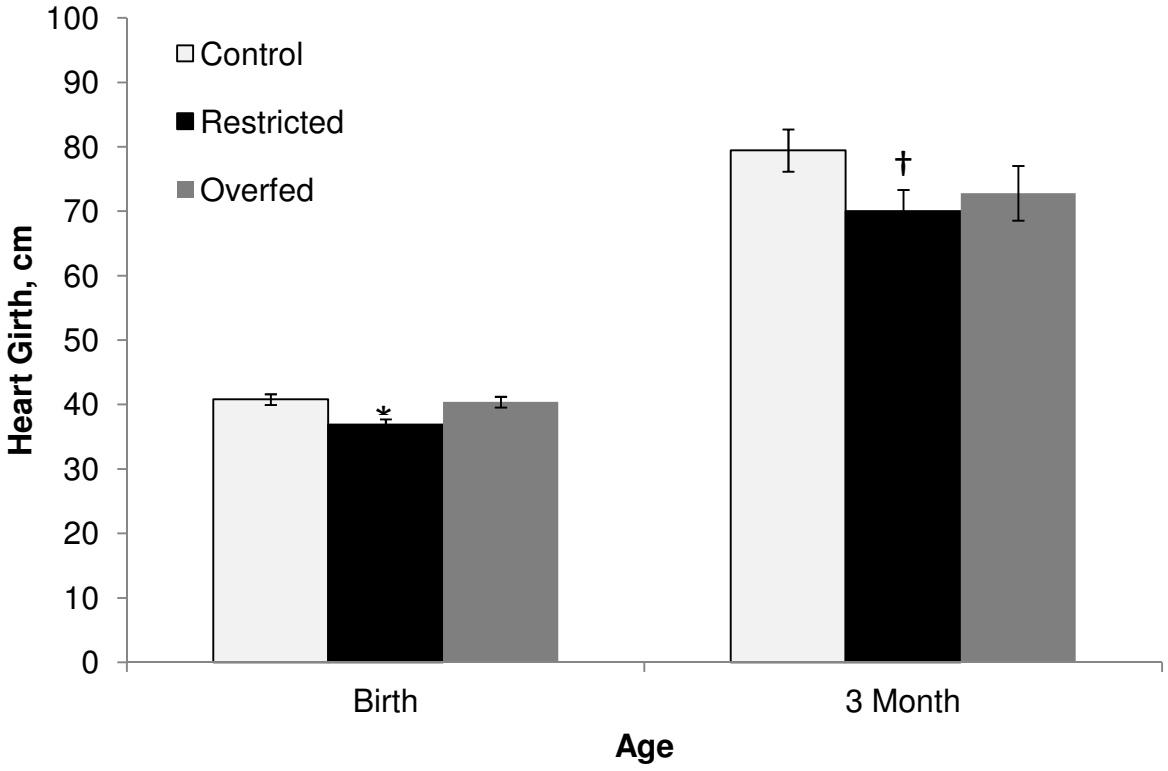

Figure 2: Heart girth circumference (cm) for all lambs at birth (n=24) and 3 months of age (n=13). * Heart girth at birth was reduced by 9% in res-lambs compared with con-lambs (*P□0.01*). † Heart girth was reduced by 12.5% for res-lambs compared with the con-lambs at three months of age (*P=0.07*). SEM = 0.818 at birth; SEM = 2.39 at 3 months.

Crown Rump Length

Crown rump length at birth averaged 47.18 ± 1.59 cm, 49.37 ± 1.59 cm, and 50.87 ± 1.59 cm for res-lambs, con-lambs and over-lambs (*P=0.27;* Figure 3), respectively. Overall, crown rump length increased by an average of 47.24 cm for all lambs. Con-lambs were not different compared with res-lambs (*P=0.34)* and over-lambs (*P=0.51)*. Con-lambs increased by 48.28 cm, res-lambs by 46.64 cm, and over-lambs by 46.80 cm. (*P=0.27*; Figure 3)

At three months crown rump length averaged 93.82 ± 1.80 cm, 97.65 ± 1.80 cm, and 95.68 ± 2.33 cm for res-lambs, con-lambs and over-lambs, respectively and was not different between treatment (*P=0.2*9; Figure 3).

Heart Weight and Liver Weight

Heart weight and liver weight were quantified for lambs in the birth slaughter group and the three month slaughter group. Heart weight at birth was 30.26 ± 3.26 g, 36.32 ± 3.26 g, and 43.88 ± 3.26 g for res-lambs, con-lambs, and over-lambs (*P=0.06*; Figure 4a), respectively. Res-lambs heart weight was reduced by 18% (13.62 g) compared with over-lambs (*P=0.02*), but heart weight was similar between con-lambs and res-lambs (*P=0.15)* or over-lambs (*P=0.23)*. Heart weight for over-lambs was increased, 7.56 g, when compared with con-lambs by (*P=0.15).* When a covariate was used to adjust for body weight res-lambs compared with con-lambs had a similar heart weight (*P=0.42),* whereas over-lambs compared with con-lambs had an increase

Figure 3: Average Crown Crump Length, for Lamb at Birth and Three Months of Age

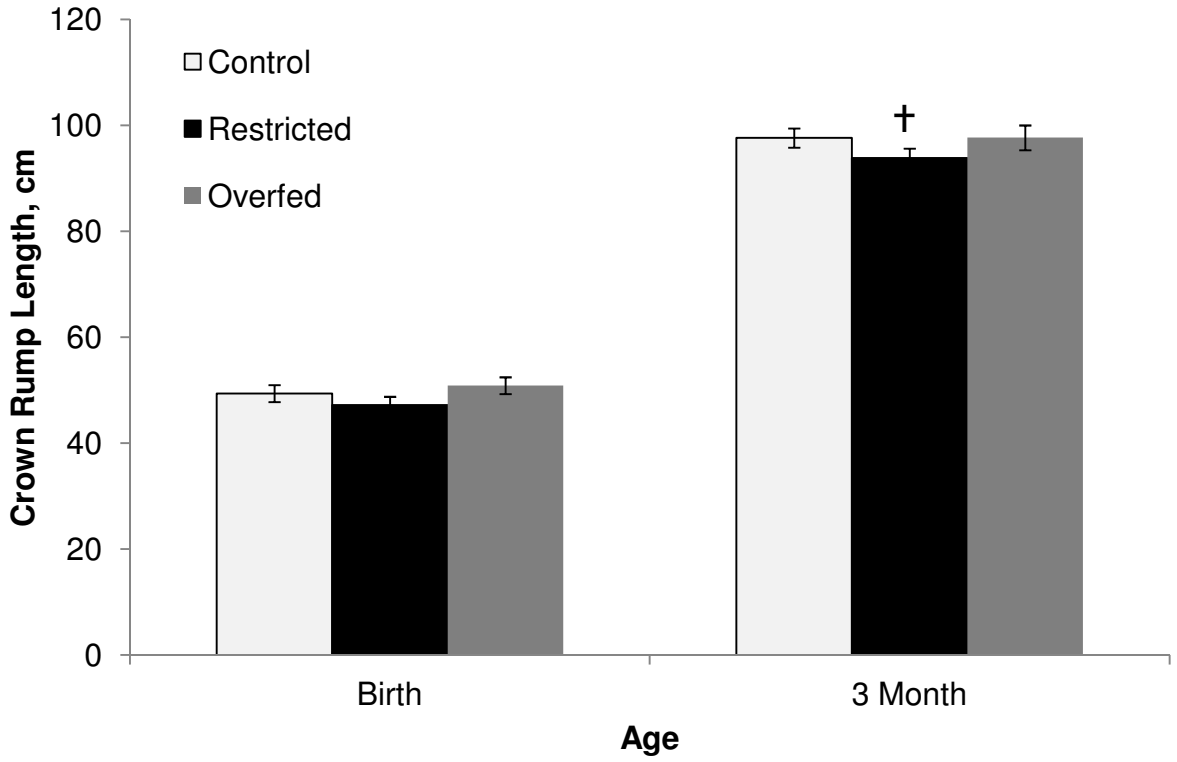

Figure 3: Crown rump length in centimeters for all lambs at birth (n=24) and 3 months of age (n=13). Crown rump length at birth was similar (*P=0.27)*. † At 3 months res-lambs had a 4% (3.83 cm) shorter crown rump length compared with con-lambs (*P=0.16*). SEM = 1.56 at birth SEM = 1.88 at 3 months.

in heart weight (*P=0.09)*. At three months of age con-lambs, res-lambs, and over-lambs had an average heart weight of 170 ± 11.42 g, 153.64 ± 11.42 g, and 159.09 ± 14.75 g (Figure 4b; *P=0.60),* respectively. Heart weight for res-lambs and over-lambs were similar to con-lambs at three months of age (*P=0.33; P=0.57)*, respectively. When a covariate was used to adjust for body weight res-lamb and over-lambs were similar to con-lambs heart weight (*P=0.24, P=0.92)*, respectively.

Average liver weight at birth was 99.88 ± 19.94 g, 116.53 ± 19.94 g, and 119.55 ± 19.94 g for res-lambs, con-lambs, and over-lambs (*P=0.76,* Figure 5a), respectively. When a covariate was used to adjust for body weight Res-lambs and over-lambs compared with con-lambs had a similar heart weight (*P=0.31; P=0.40)*, respectively. At three months of age liver weight for con-lambs, res-lambs, and over-lambs averaged 623.64 ± 38.22 g, 594.55 ± 38.22 g, and 616.67 ± 49.35 g, respectively and was not different between treatment (*P=0.85,* Figure 5b*)*. When a covariate was used to adjust for body weight over-lambs were similar to con-lambs liver weight (*P=0.30)*. While res-lambs when compared to con-lambs had a larger liver weight when adjusted for body weight (*P=0.05)*.

Figure 4a: Heart Weight vs Body Weight for Lambs at Birth

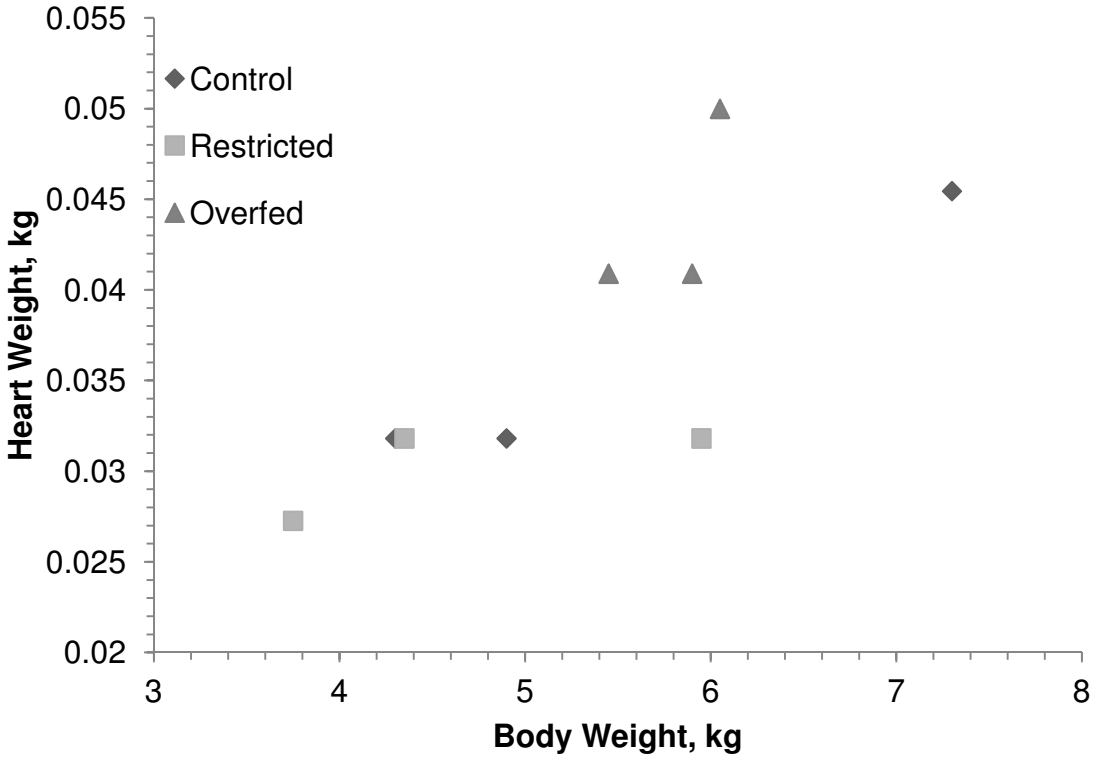

Figure 4a: Heart weight compared with body weight of lambs at birth (n=9). Heart weight at birth averaged 30.26 ± 3.26 g, 36.32 ± 3.26 g, and 43.88 ± 3.26 g for res-lambs, con-lambs, and over-lambs, respectively. SEM = 3.03. When adjusted for body weight over-lambs had a heavier heart compared with con-lambs (*P=0.09)*.

Figure 4b: Heart Weight vs Body Weight for Lambs at Three Months

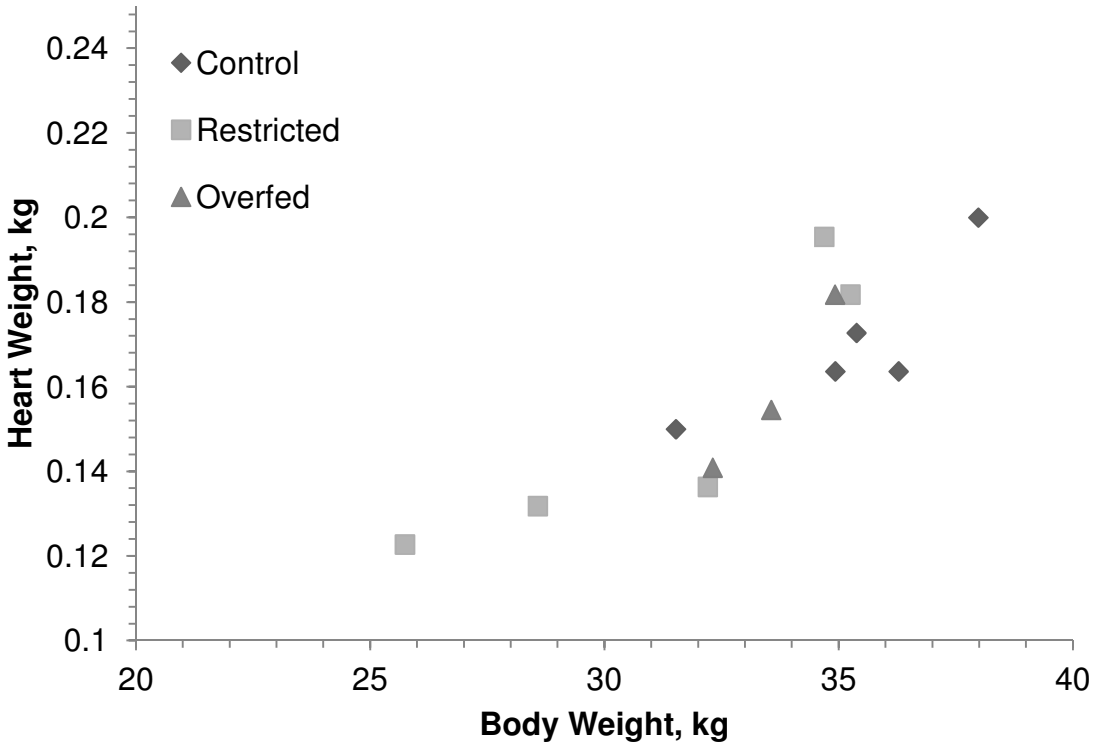

Figure 4b: Heart weight compared with body weight for lambs at three months of age (n=13). Heart weight averaged 152.64 ± 11.42 g, 170 ± 11.42 g, and 159.09 ± 14.75 g for res-lambs, con-lambs and over-lambs (*P=0.60),* respectively. SEM = 11.65. When adjusted for heart weight the treatments were similar.

Figure 5a: Liver Weight vs Body Weight for Lambs at Birth

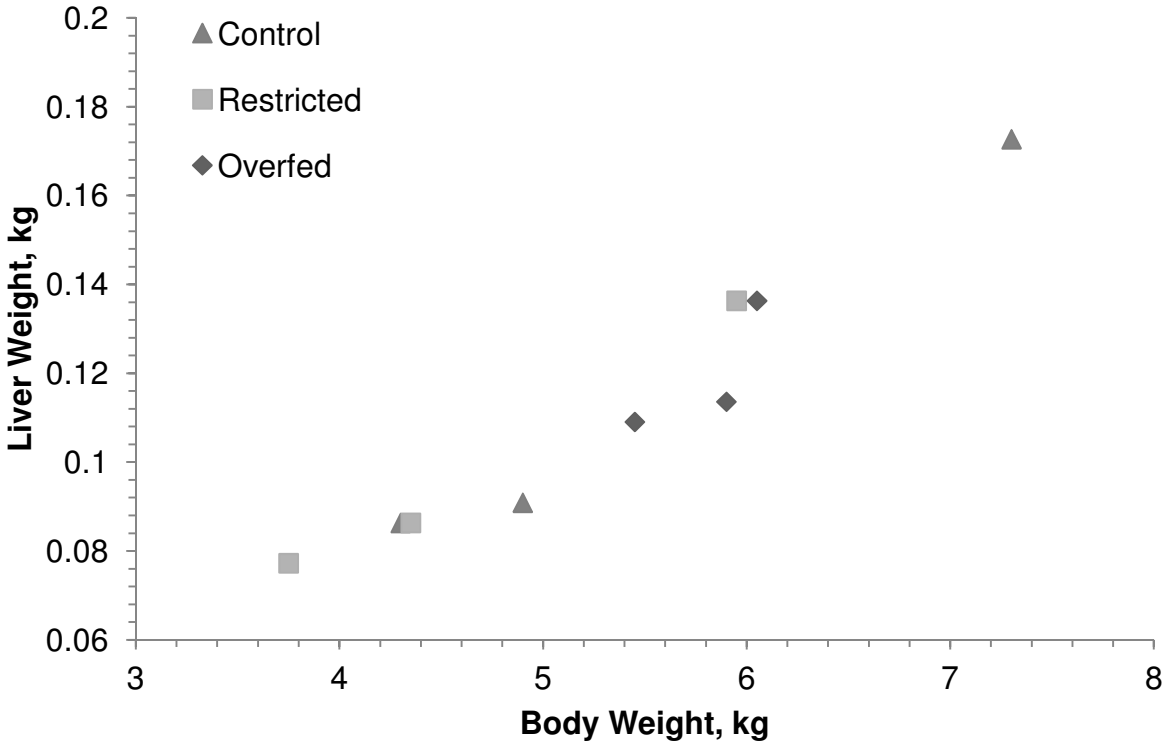

Figure 5a: Liver weight compared with body weight of lambs at birth (n=9) and three months of age (n=13). Liver weight at birth averaged 99.88 ± 19.94 kg, 116.53 ± 19.94 kg, and 119.55 ± 19.94 kg for res-lambs, con-lambs and over-lambs, respectively. SEM = 18.28. When adjusted for body weight res-lambs and over-lambs were similar compared with con-lambs (*P=0.40, P=0.31)*.

Figure 5b: Liver Weight vs Body Weight for Lambs at Three Months

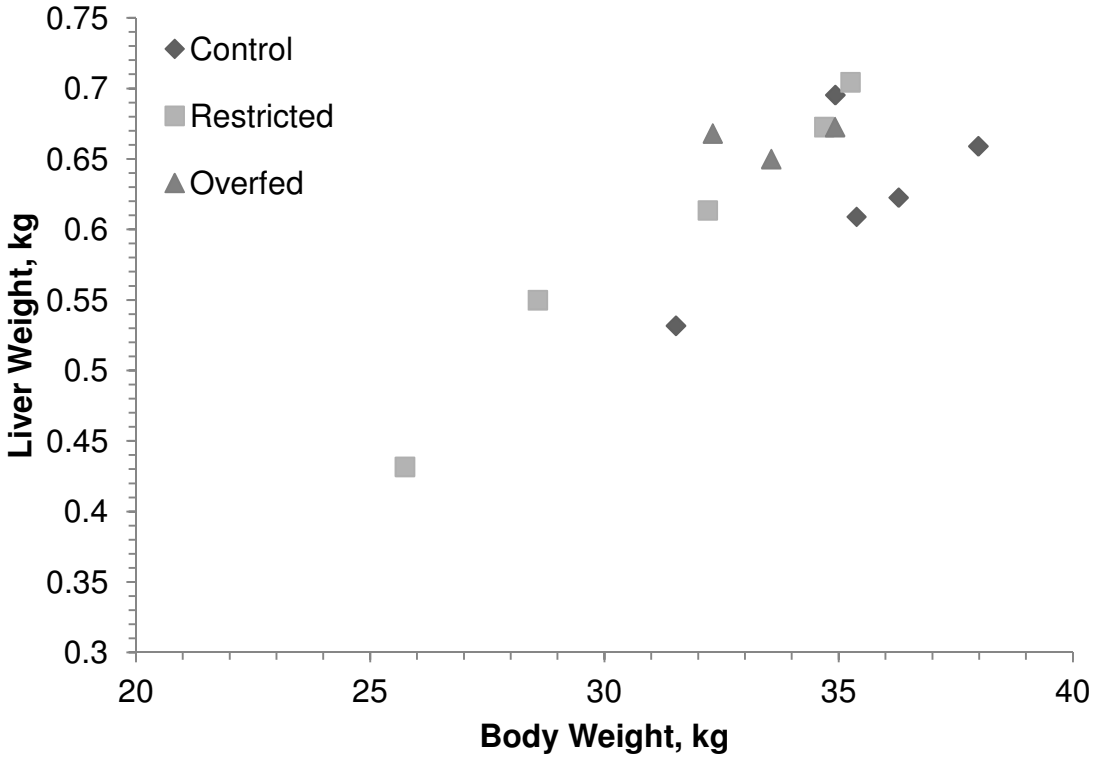

Figure 5b: At three months liver weight compared with body weight at three months of age (n=13). Liver weight averaged 594.55 ± 38.22 kg, 623.64 ± 38.22 kg, 616.67 ± 49.35 kg for res-lambs, con-lambs and over-lambs (*P=0.85),* respectively. SEM = 36.34. When adjusted for body weight over-lambs were similar compared with con-lambs (*P=0.30)*. Res-lambs had a larger liver when adjusted for body weight (*P=0.05)*.

Backfat Thickness and Loin-eye Area

Backfat thickness at three months of age was 1.4 ± 0.33 mm, 2.8 ± 0.33 mm, and 2.5 ± 0.42 mm for re-lambs, con-lambs and over-lambs respectively. Res-lambs compared to con-lambs had 66% less backfat (*P=0.01).* Backfat thickness as a percentage of body weight at three months of age averaged 4.4 ± 0.8%, 7.9 ± 0.8%, and 7.4 ± 1.1% for res-lambs, con-lambs, and over-lambs (*P=0.03;* Figure 6), respectively. Con-lambs had 56% more backfat then res-lambs (*P=0.01)*, whereas over-lambs had 50% more backfat compared with res-lambs (*P=0.05)*. Over-lambs compared with con-lambs had a similar amount of backfat (*P=0.74).*

Loin eye area at three months of age was 25.2 cm^2, 27.9 cm^2 and 25.2 cm^2 for res-lambs, con-lambs, and over-lambs, respectively. three months of age loin eye area, which was quantified as a percentage of body weight, averaged 83.00 ± 4.6%, 79.35 ± 4.6%, and 75.35 ± 5.9% for res-lambs, con-lambs, and over-lambs, respectively and was not different between treatments (*P=0.61;* Figure 7*)*.

Growth Hormone

Growth Hormone averaged across one week of age to three months of age was 1.99 ± 0.30 ng/mL, 1.49 ± 0.35 ng/mL, and 1.95 ± 0.35 ng/mL for res-lambs, con-lambs, and over-lambs (*P=0.51;* Figure 8), respectively. Con-lambs compared with res-lambs or over-lambs were similar over the time between week one and three months of age (*P=0.28; P=0.35),* respectively. Growth hormone at birth averaged 2.61 ± 0.65 ng/m, 1.19 ± 0.65 ng/mL, and 2.12 ± 0.72 ng/mL, for res-lambs, con-lambs, and over-

Figure 6: Backfat at Three Months of age

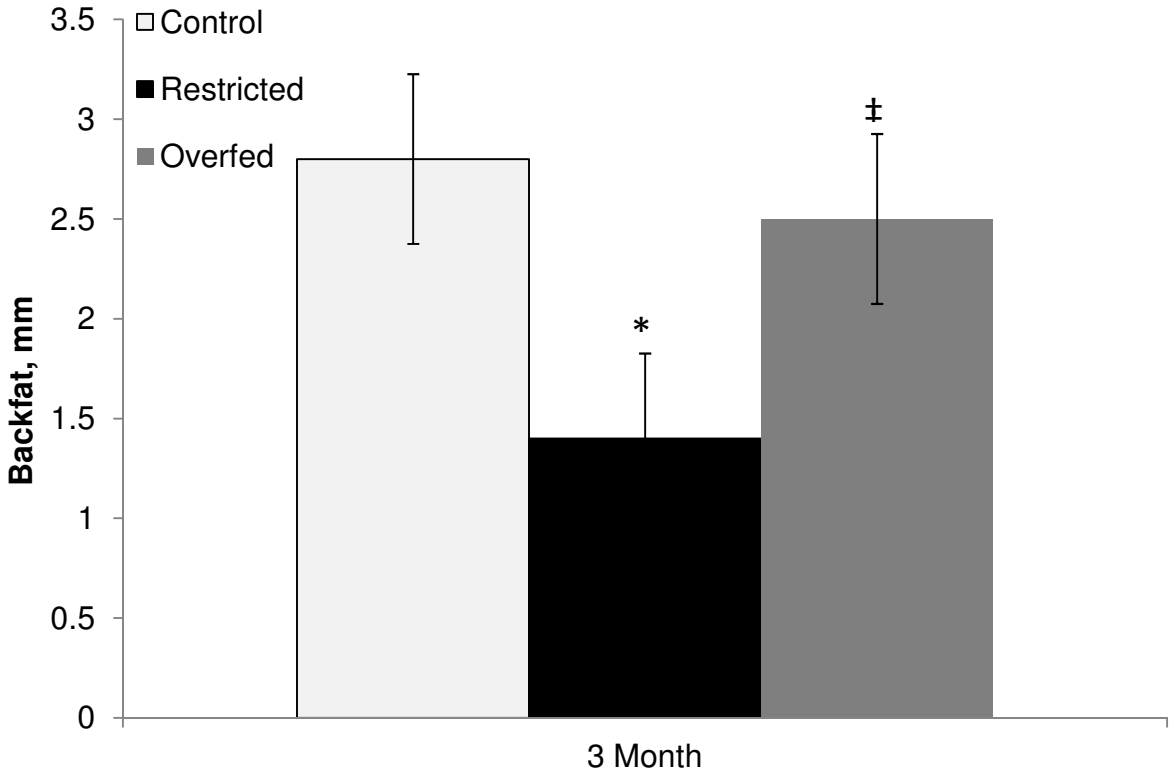

Figure 6: Backfat measured in millimeters for lambs of each of the maternal treatment groups (restricted, control and overfed) at three months of age (n=13). * Res-lambs had 66% less (1.4 mm) backfat compared with con-lambs (*P=0.01*). ‡ Over-lambs had 11% less, 0.3 mm backfat compared with over-lambs (*P=0.05*).

Figure 7: Loin Eye Area in cm^2 at Three Months of age

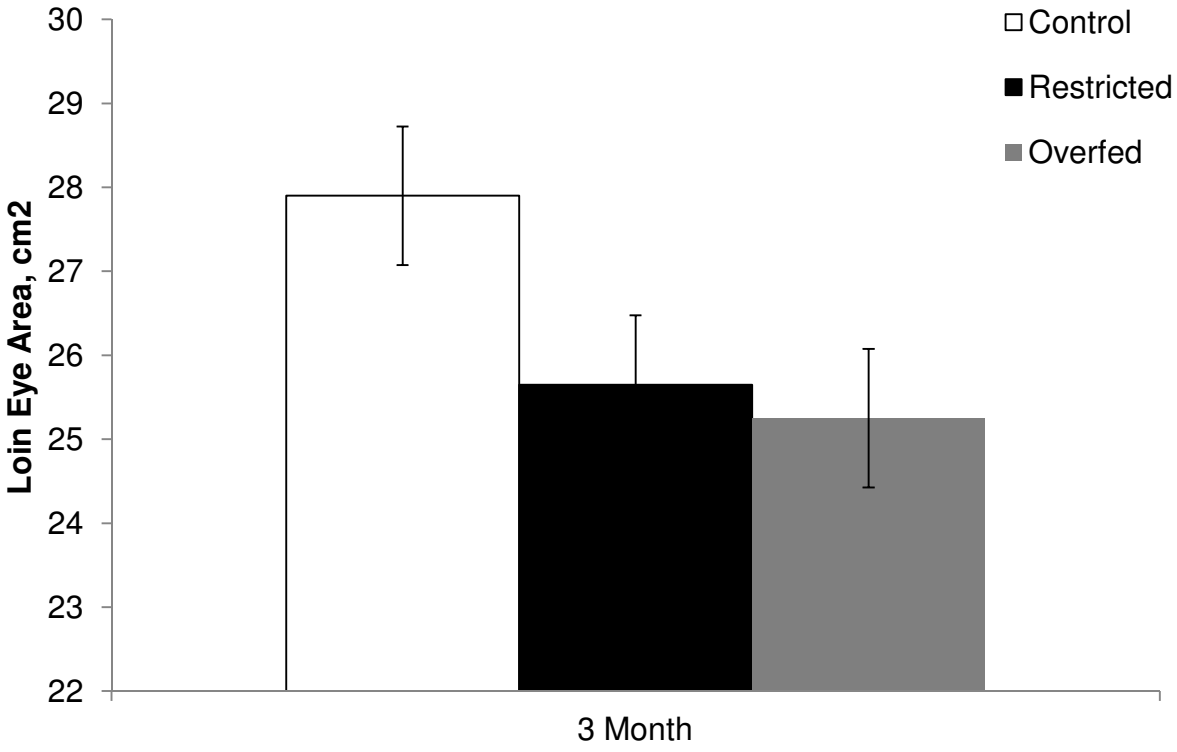

Figure 7:Loin eye area measured in centimeters squared for the lambs from each of the maternal treatment groups (restricted, control and overfed) at three months of age (n=13). There were no differences observed between treatment groups (*P=0.61*).

lambs respectively. Res-lambs and over-lambs compared to con-lambs GH concentrations were similar at one week of age (*P=0.12, P=0.34*), respectively. At three months of age the GH concentration averaged 0.98 mg/mL got res-lambs and similarly 0.83 for con-lambs (*P=0.88*).

Serum IGF-I Concentrations

Serum IGF-I concentrations for all the lambs maintained on the study between birth and three months of age was 231.51 ± 38.04 ng/mL. Insulin-like growth factor-I concentrations averaged over the entire lifespan was 158.84 ± 36.37 ng/mL, 308.16 ± 37.32 ng/mL, and 227.55 ± 40.42 ng/mL for res-lambs, con-lambs, and over-lambs (*P=0.04),* respectively. Con-lambs had 63% (149.32 ng/mL) less circulating IGF-I compared with res-lambs over the entire lifespan of the lambs (*P=0.01*). Over-lambs were similar compared with con-lambs over the entire lifespan of the lambs (*P=0.16).* At birth IGF-I concentrations averaged 158.48 ± 55.02 ng/mL, 171.02 ± 55.02 ng/mL, 172.59 ± 55.02 ng/mL for res-lambs, con-lambs, and over-lambs (*P=0.98*), respectively. At three months of age IGF-I concentrations averaged 148.09 ± 98.17 ng/mL, 300.52 ± 109.76 ng/mL, and 292.63 ± 126.74 ng/mL mL for res-lambs, con-lambs, and over-lambs, respectively and were not different between treatments (*P=0.53*; Figure 9).

Figure 8: Growth Hormone, for Lambs from Birth to Three Months of age

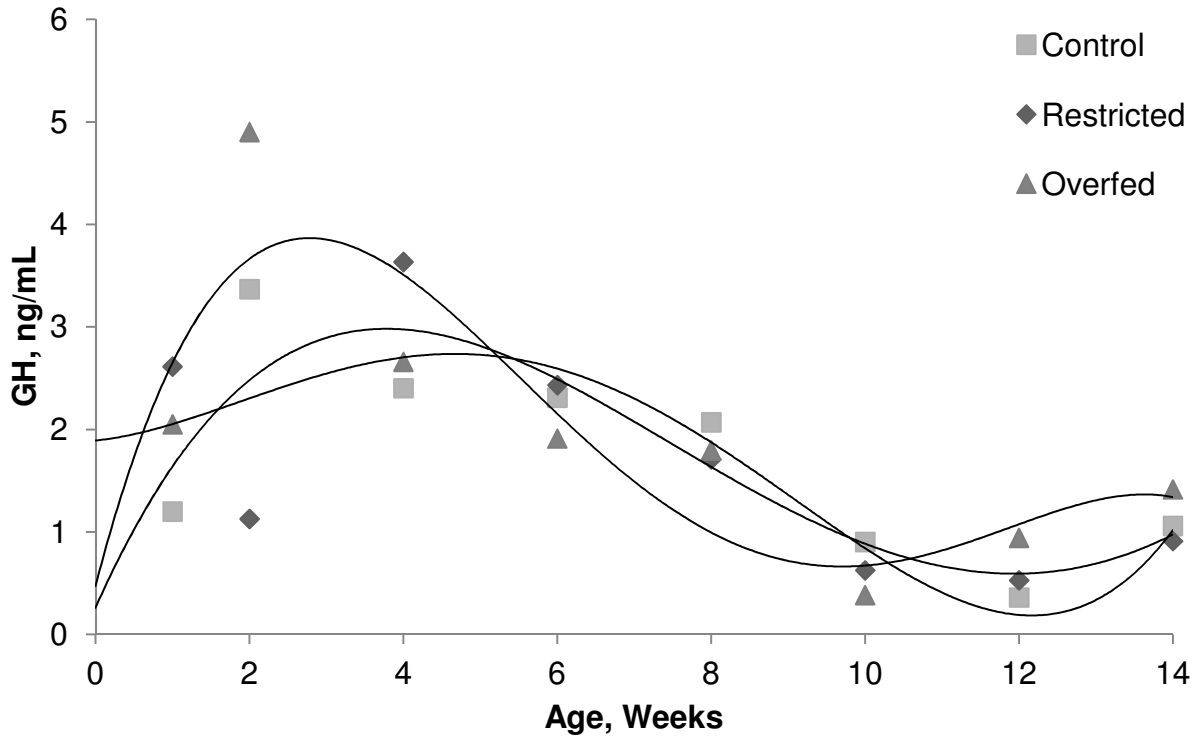

Figure 8: Growth hormone (GH) concentration of each of the maternal treatment groups (restricted, control and overfed) from birth to three months of age (n=15). Lines represent quartic regression lines for each treatment, a better fit than a linear regression line. The equation for Control = $y = -0.0003x^4 + 0.0195x^3 - 0.3231x^2 + 1.6805x + 0.2599$, $R^2 = 0.7682$; Restricted = $y = 0.001x^4 - 0.0228x^3 + 0.1067x^2 + 0.0752x + 1.8916$, $R^2 = 0.6755$; Overfed = $y = -0.002x^4 + 0.0682x^3 - 0.773x^2 + 2.885x + 0.4705$, $R^2 = 0.7366$. Average GH for the entire experiment were 1.99 ± 0.30 ng/mL, 1.49 ± 0.35 ng/mL and 1.95 ± 0.35 ng/mL for res-lambs, con-lambs, and over-lambs (*P=0.51),* respectively.

Figure 9: Insulin-like Growth Factor-1, for Lambs from Birth to Three Months of age

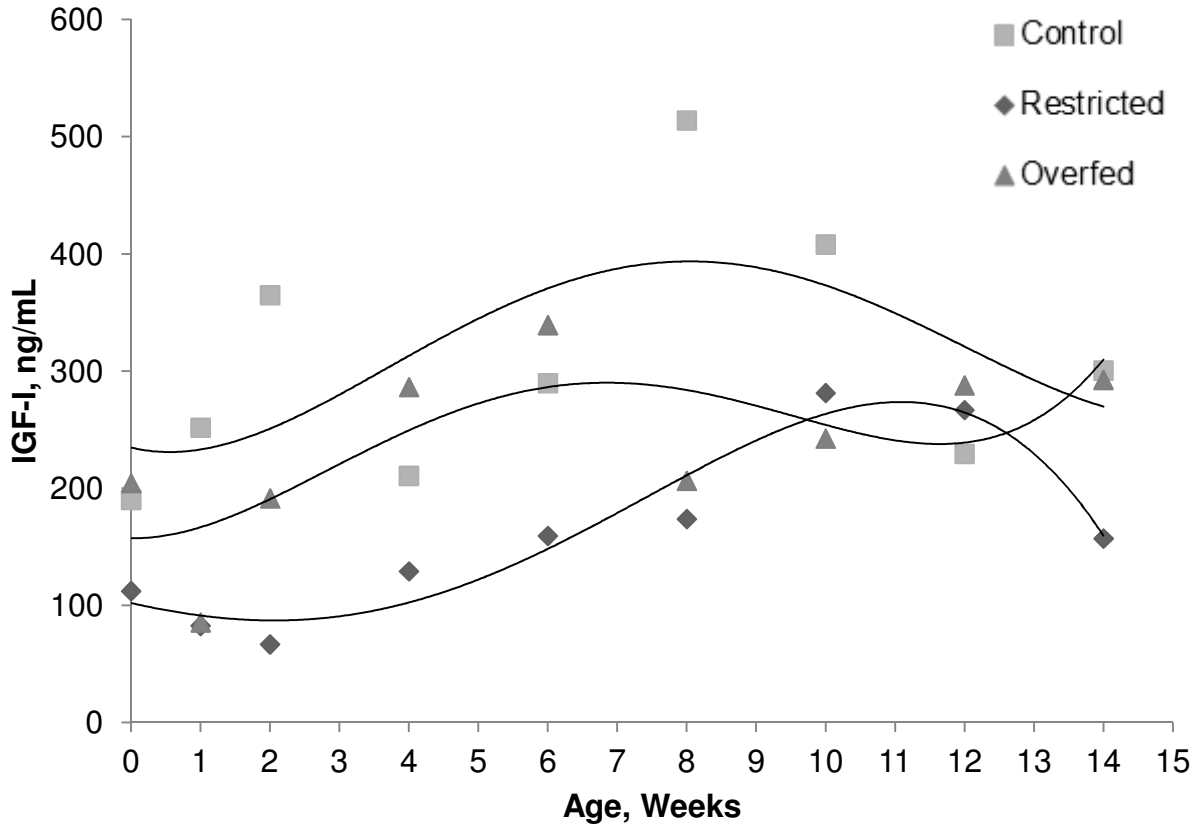

Figure 9: Insulin-like growth factor-1 (IGF-I) concentration of each of the maternal treatment groups (restricted, control and overfed) averaged over the entirety of the study from birth to three months of age (*P=0.04*; *n*=15). Lines represent quartic regression lines for each treatment, a better fit than a linear regression line. The equation for Control = $y = 0.0543x^4 - 1.7088x^3 + 14.498x^2 - 14.587x + 234.76$, $R^2 = 0.3534$; Restricted = $y = -0.0359x^4 + 0.437x^3 + 2.1498x^2 - 13.174x + 101.96$, $R^2 = 0.9288$; Overfed = $y = 0.0774x^4 - 1.9145x^3 + 12.507x^2 - 1.3555x + 157.36$, $R^2 = 0.5221$. Insulin-like growth factor-I averaged 158.8 ± 36.3 ng/mL, 308.1 ± 37.3 ng/mL, and 227.5. ± 40.42 ng/mL for res-lambs, con-lambs, and over-lambs (*P=0.04),* respectively. Res-lambs had 63% (149.32 ng/mL) less IGF-I compared with con-lambs (*P=0.01*). Over-lambs, had 30% (80.61 ng/mL) less IGF-I compared with con-lambs (*P=0.16*). SEM = 46.77

Serum IGFBP-3 Concentration

Overall, IGFBP-3 averaged for the entire experiment was 1,032 ± 133 AU, 1,438 ± 135 AU, and 1,014. ± 148 AU for res-lambs, con-lambs, and over-lambs, respectively (*P=0.05).* Insulin-like growth factor binding protein-3 at birth averaged 547.26 ± 52.75 AU, 597.65 ± 52.75 AU, and 638.07 ± 52.75 AU at birth for res-lambs, con-lambs, and over-lambs, respectively and there were no differences between treatments (*P=0.48;* Figure 10). At three months of age, IGFBP-3 averaged 1,252.47 ± 442.87 AU, 2,221.73 ± 442.87 AU and 2,034.27 ± 571.74 AU for res-lambs, con-lambs, and over-lambs, respectively with no difference between treatments (*P=0.31*). Res-lambs had 32% (406 AU) less IGFBP-3, compared with con-lambs (*P=0.03).* Over-born lambs had 36% (424 AU) less IGFBP-3 compared with con-lambs (*P=0.03*; Figure 10). Although each the two time points, birth and three months of age were similar, the overall effect reflects a treatment vs. time interaction.

Serum IGFBP-2 Concentration

Insulin-like growth factor binding protein-2 at birth averaged 255.3 ± 28.47 AU, 242.83 ± 28.47 AU, and 266.97 ± 28.47 for res-lambs, con-lambs, and over-lambs (*P=0.83;* Figure 11), respectively. Average IGFBP-2 was similar between treatments for the entire experiment with 214.31 ± 16.58 AU, 192.81 ± 16.58 AU, and 201.58 ± 17.18 AU for res-lambs, con-lambs, and over-lambs, respectively (*P=0.63).*

Figure 10: Insulin-like Growth Factor Binding Protein-3 in Arbitrary Units, for Lambs from Birth to Three Months of age

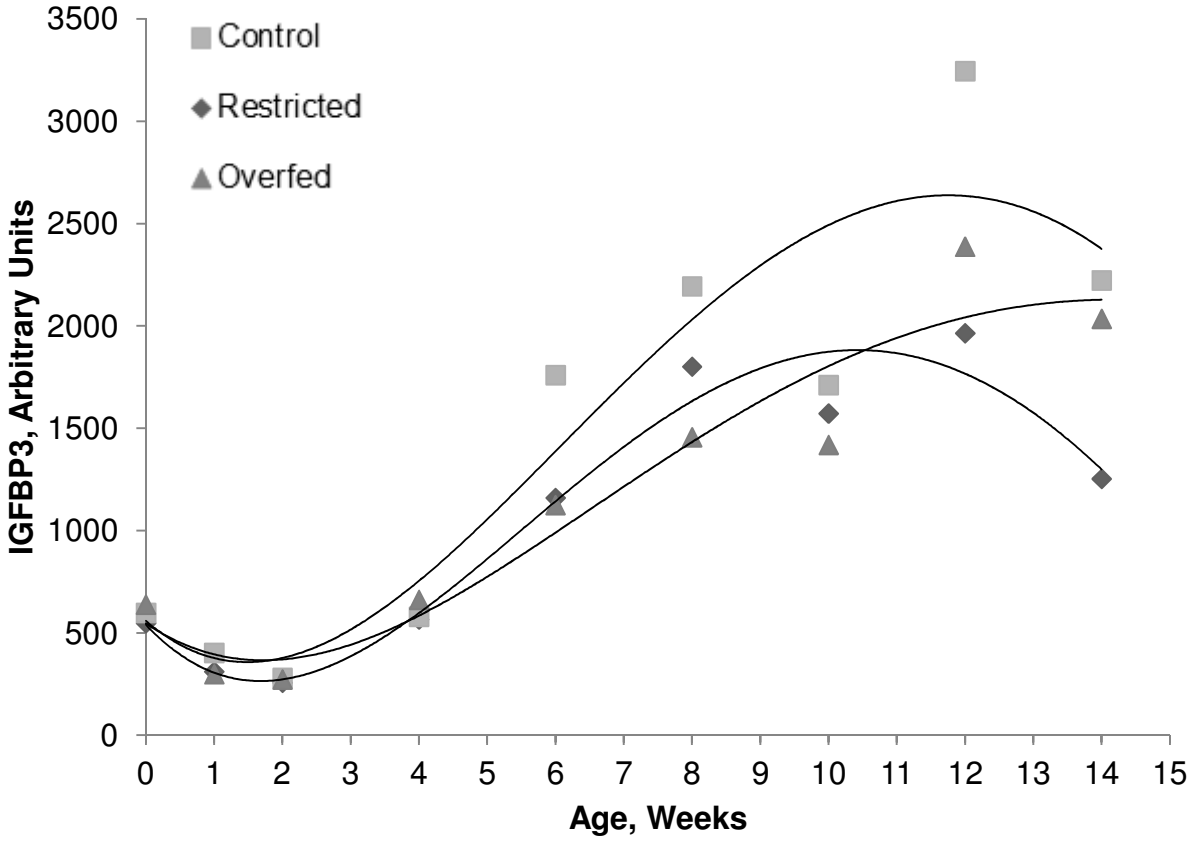

Figure 10: Insulin-like growth factor binding protein-3 (IGFBP-3) concentrations for each of the maternal treatment groups (restricted, control and overfed) from birth to three months of age (n=15). Lines represent quartic regression lines for each treatment. The equation for Control = $y = 0.1415x^4 - 7.9619x^3 + 113.47x^2 - 286.63x + 558.2$, $R^2 = 0.8569$; Restricted = $y = 0.2318x^4 - 10.457x^3 + 130.11x^2 - 353.75x + 539.63$, $R^2 = 0.9521$; Overfed = $y = 0.1178x^4 - 5.5527x^3 + 78.712x^2 - 223.9x + 545.88$, $R^2 = 0.9265$. Overall, IGFBP-3 averaged was 1,032 ± 133 AU, 1,438 ± 135 AU, and 1,014. ± 148 AU for res-lambs, con-lambs, and over-lambs (*P=0.05),* respectively for the entire experiment. Res- lambs had 32% (406 AU) less IGFBP-3 compared with con- lambs (*P=0.03).* Over-lamb had 36% (424 AU) less IGFBP-3 compared with con-lambs (*P=0.*03). SEM = 209

Figure 11: Insulin-like Growth Factor Binding Protein-2, for Lambs from Birth to Three Months of age

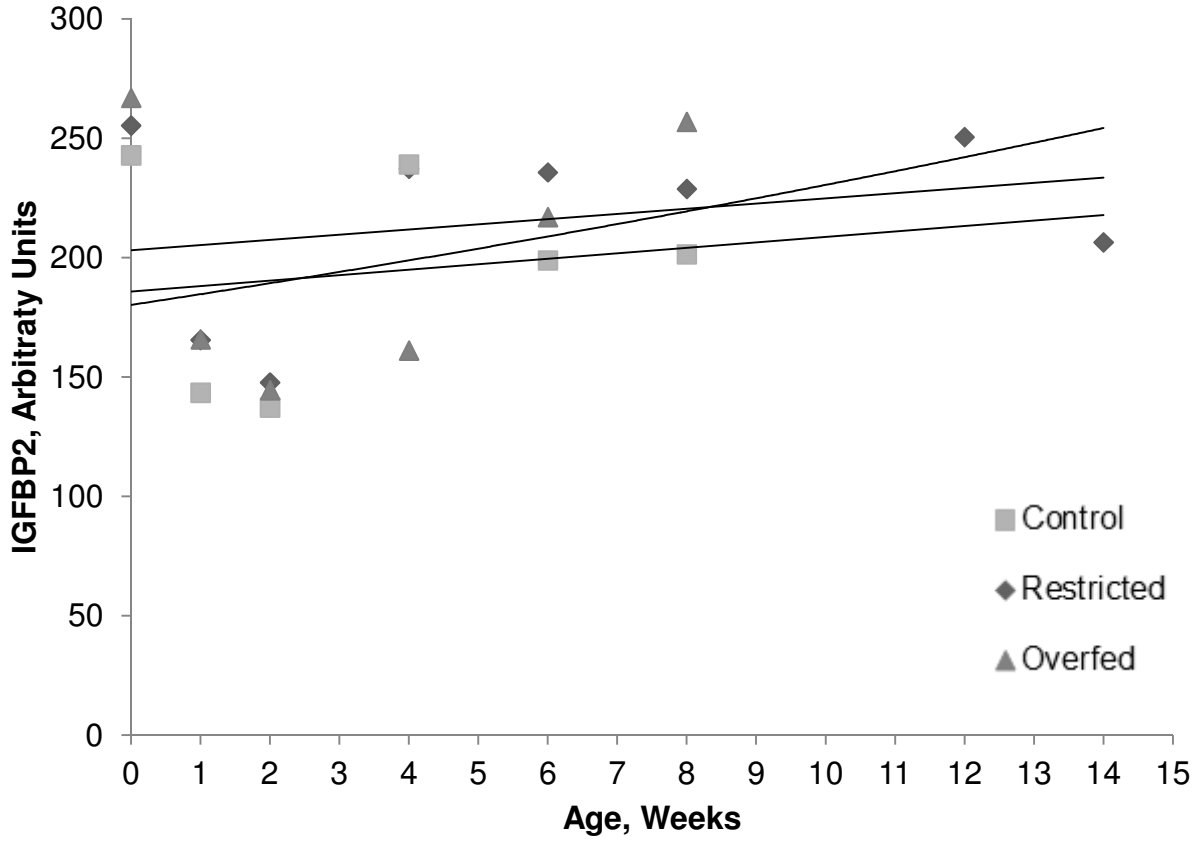

Figure 11: Insulin-like growth factor binding protein-2 (IGFBP-2) concentration for each of the maternal treatment groups (restricted, control and overfed) from birth to three months of age (n=15). Lines represent logarithmic regression lines; res-lambs y=4.49x+186.3, con-lambs y=2.28x+185.79, and over-lambs y=2.17x+203.09. Average IGFBP-2 for the entire experiment were 214.31 ± 16.58 AU, 192.81 ± 16.58 AU, and 201.58 ± 17.18 AU for res-lambs, con-lambs, and over-lambs (*P=0.63),* respectively.

Thyroid Hormones

At birth T3 averaged 5.56 ± 0.12 ng/mL, 5.95 ± 0.12 ng/mL, 5.94 ± 0.12 ng/mL for res-lambs, con-lambs, and over-lambs (*P=0.08;* Figure 12), respectively. At birth, res-lambs had 6% less T3 compared with con-lambs (*P=0.04)*. At three months of age T3 concentrations were similar between treatment groups when averaged at 3.38 ± 0.31 ng/mL, 2.98 ± 0.31 ng/mL, and 2.87 ± 0.36 ng/mL for res-lambs, con-lambs, and over-lambs, respectively (*P=0.53*).

At birth T4 averaged 14.88 ± 0.75 ng/mL, 16.28 ± 0.75 ng/mL, 16.44 ± 0.75 ng/mL for res-lambs, con-lambs, and over-lambs, respectively, and were similar between treatments (*P=0.29;* Figure 13). At three months of age T4 averaged 6.52 ± 1.12 ng/mL, 7.19 ± 1.00 ng/mL, and 5.95 ± 1.30 for res-lambs, con-lambs, and over-lambs, respectively, were similar between treatments (*P=0.75*).

Figure 12: Triiodothyronine (T3), for Lambs at Birth and Three Months of age

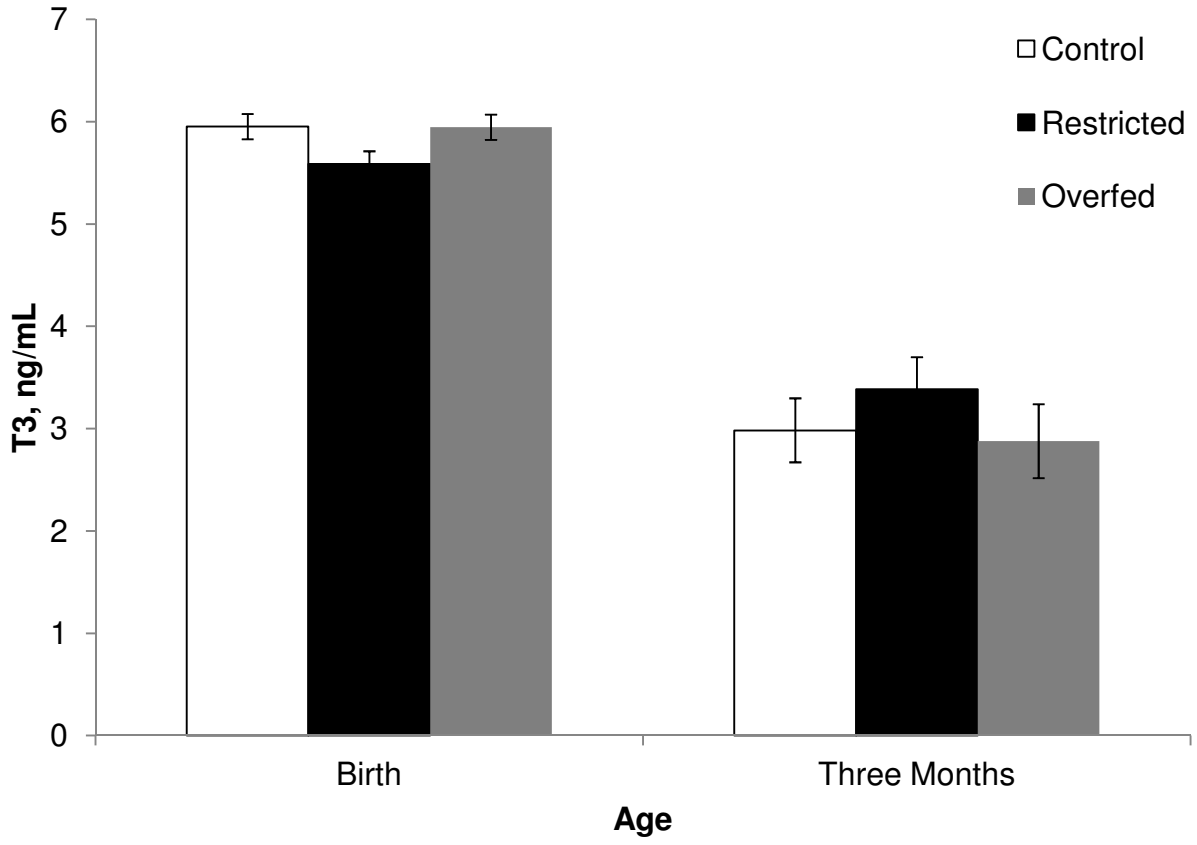

Figure 12: Average triiodothyronine (T3) concentration for each maternal treatment group (restricted, control and overfed) at birth (n=24) and three months of age (n=11). Average T3 at birth was 5.58 ± 0.12 ng/mL, 5.95 ± 0.12 ng/mL and 5.94 ± 0.12 ng/mL for res-lambs, con-lambs, and over-lambs (*P=0.08),* respectively. Average T3 at three months was 3.38 ± 0.31 ng/mL, 2.98 ± 0.31 ng/mL and 2.87 ± 0.36 ng/mL for res-lambs, con-lambs, and over-lambs (*P=0.53),* respectively.

Figure 13: Thyroxine (T4), for Lambs at Birth and Three Months of age

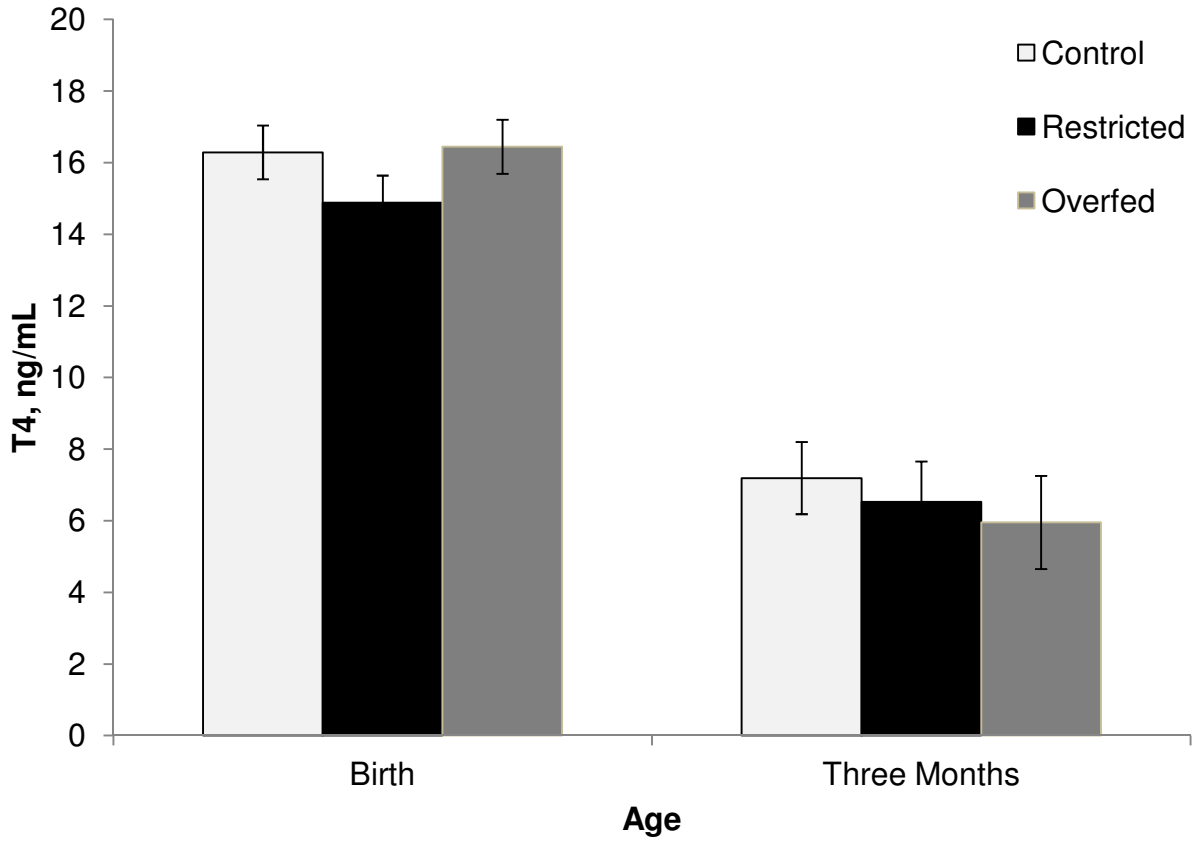

Figure 13: Average Thyroxine (T4) concentration for each maternal treatment group (restricted, control and overfed) at birth (n=24) and three months of age (n=12). Average T4 at birth was 14.88 ± 0.75 ng/mL, 16.28 ± 0.75 ng/mL and 16.44 ± 0.75 ng/mL for res-lambs, con-lambs, and over-lambs (*P=0.29),* respectively. Average T4 at three months was 6.52 ± 1.12 ng/mL, 7.19 ± 1.00 ng/mL and 5.95 ± 1.30 ng/mL for res-lambs, con-lambs, and over-lambs (*P=0.75),* respectively.

Discussion

This study was conducted to determine the effects of poor maternal nutrition during late gestation on both prenatal and postnatal lamb growth, indicators of body composition, organ weight, concentrations of the somatotropic hormones (GH, IGF-I, IGFBP-3, and IGFBP-2), and concentrations of T3 and T4. In utero, a fetus experiencing poor maternal nutrition will optimize its developmental path to promote postnatal survival (Khan et al., 2004). However, when the postnatal environment fails to meet the expectations and adaptions of the offspring, instead of promoting survival, alternatively disease and growth imbalances (greater fat deposition vs. muscle growth) will be the result (Khan et al., 2004). Poor maternal nutrition constitutes both overfeeding and underfeeding of the mother (Wu et al., 2006). Both of which have negative implications for the offspring (Wu et al., 2006). Many poor maternal nutrition studies focused on placental insufficiency, restricting ewe nutrition, or overfeeding to the point of obesity (Vonnahme et al., 2008; Reynolds et al., 2010, Long et al., 2011). The current study focuses and expands upon the current literature in combination with the knowledge that in ruminants there is a connection between nutritional status, and circulating GH and IGF-I concentrations (McGuire et al., 1992; Lutz et al., 2006). A key focus of the present study was the hormones and binding proteins of the somatotropic axis in lambs and how they respond to changes in maternal nutrition. In the current study the most pronounced effects of the maternal diet on the progeny were observed in body weights and serum concentrations of IGF-I and IGFBP-3 of the lambs.

Restricting nutritional intake of ewes resulted in lambs that were lighter at birth compared with lambs born to control-fed ewes. As hypothesized, the restricted lambs remained smaller than the control born lambs for the duration of the experiment. On average these lambs were 16% lighter than age matched controls. Similar to the results that were observed in the current study, Sebert et al. (2011) reported a difference in birth weights of lambs born to restricted fed ewes. Ewes were restricted to 60% of NRC requirements, the same degree as the current study, resulting in lambs that were lighter at birth (Sebert et al., 2011). Tygesen et al. (2007) also observed a reduction in body weight in lambs born to ewes fed 60% of NRC requirements. Lambs born to these ewes weighed 0.9 kg less than lambs born to control-fed ewes (Tygesen et al., 2007). This decrease in birth weight supports the hypothesis that restricting the maternal nutrition results in smaller lambs at birth. No differences were observed in birth weights between lambs born to control-fed ewes and ewes fed 50% NRC from day 31 of gestation until parturition (Khan et al., 2004). Khan et al. (2004) postulated that the lack of differences observed was due to birth weight and body vaiables being inconsistent indicators of fetal nutrition (Khan et al., 2004). This lack of response in the lambs to the maternal nutrient restriction could have been due, in part, to compensatory mechanisms, such as changes genomic expression overriding the lack of nutrients. At three months of age, restricted born lambs in the current study were consistently smaller than controls by 12%. To test the downstream effects of maternal nutrition beyond three months of age, Sebert et al. (2011) fed ewes 60% of NRC requirements from day 110 of gestation and maintained the lambs until either 3 or 7 months of age. These data indicated acceleration in body weight gain beginning at three months of age, resulting in body

weights similar to age-matched controls by seven months of age (Sebert et al., 2011). This change in growth rate was the result of changes to postnatal energy homeostasis brought on by decreased maternal nutrition (Sebert et al., 2011).

Lambs that were born to ewes that were in the overfed treatment group were not different in terms of body weight at birth from the lambs born to control-fed ewes. This is similar to Peel et al. (2012), who reported that ewes who consumed 122% of NRC requirements during mid to late gestation produced lambs that had similar body weights at birth to lambs born to control-fed ewes. Unique to the current study was the time-dependent differences in lambs born to overfed ewes compared with control-fed ewes. From birth to 6 weeks of age there was no difference between these two treatment groups. Whereas between weeks 7 and 8 overfed lambs were substantially smaller than control born lambs by 3.33 kg and 3.42 kg, respectively. The lambs born to overfed ewes had accelerated body weight gain from 9 weeks to three months of age, resulting in lambs whose body weight was not different when compared with control born lambs. This increase in body weight occurs post-weaning, which could correlate to difficulties in the lambs adapting to an ad libitium diet. It would be worth investigating leptin concentrations to see if the appetite control center of the brain was impacted by the maternal diet (Monteleone and Maj, 2013).

There was a 10% reduction (3.89 cm) in heart girth circumference in lambs born to restricted-fed ewes compared with control-fed born lambs. This reduction supports that the lambs that were lighter in body weight had reduced body size variables which is similar to results obtained from other IUGR studies (Louey et al., 2000). Intrauterine

growth retarded lambs had a 14% smaller heart girth circumference compared with non-IUGR lambs (Louey et al., 2000). At 8 weeks of age the IUGR lambs were still smaller, having a smaller heart girth circumference compared with control lambs by 11% (Louey et al., 2000). In the present study, at three months of age the lambs born to restricted-fed ewes had a 12.5% smaller heart girth, which supports the concept that IUGR lambs are smaller than control born lambs. Goats that were restricted to 70% of NRC requirements in the last third of their pregnancy produced kids who had a similar heart girth circumference compared with controls (Laporte-Broux et al., 2011).

It was hypothesized in the present study, that when heart girth or body weight are reduced, the crown rump length of the lambs would be reduced compared with controls. In the present study, crown rump length was similar between treatment groups at birth and at three months of age. Due to the variance in measuring crown rump, consistency is hard to maintain between measurements and between researchers taking the measurements. Louey et al. (2000), reported that IUGR lambs at birth had a shorter crown rump length, by 7% and 6%, at week 7 and 8, respectively. Meyer et al. (2010) reported that lambs born to ewes restricted to 60% NRC requirements from day 40 of gestation had a reduced crown rump length compared with control-fed ewes at birth. Compared with the current experiment Meyer et al. (2010) targeted an earlier period of gestation, therefore differences observed between experiments could be due to the difference in restriction in late gestation (last third of gestation) vs early to mid-gestation (first and second third of gestation). Neville et al. (2010) reported that ewes fed 60% of NRC from day 50 of gestation had lambs with similar crown rump length to control-fed ewes. This supports the findings of the present study where treatment similarities were

observed at the same time point. At three months of age in the current study, there were similarities in crown rump length between treatments. At 180 days of age, Neville et al. (2010), observed no difference in lambs born to restricted-fed or to control-fed ewes in crown rump length. In the present study lambs were only maintained until 90 days of age, both studies support that crown rump length is not affected by maternal nutritional plane. Although it was hypothesized that crown rump length would be reduced, the lack of differences was supported in current literature.

Organ weights, heart and liver weights were quantified at birth and three months of age for lambs. A tendency for a difference in treatments was observed in heart weight at birth, but not in liver weight. However, Reed et al. (2007) reported no difference in the heart weight of neonates carried by restricted-fed ewes. The neonatal lambs used in the Reed et al. (2007) were slaughtered at 135 days of gestation; in the current study lambs were born at approximately 145 days of gestation. In the present study, heart weight was similar at 12 weeks of age. This finding agrees with Daniel et al. (2007) who reported that 17-week old lambs born to ewes restricted between day 30 and 85 of gestation had similar heart weights between treatment groups. The data from lambs slaughtered at 17 weeks corresponds with the data reported in the present study, which in-turn corresponds to the conclusion that heart weight is not affected by maternal nutritional status. A decrease in liver weight was reported by Reed et al. (2007), in lambs born to restricted-fed ewes compared with control-fed ewes. Similar to the current study, McMullen et al. (2005) observed no differences in liver weight at day 135 of gestation for lambs whose mothers were nutrient restricted. In addition, Zhang et al. (2011) reported no differences in fetal heart or liver weight at day 135 of gestation for

lambs born to obese mothers. These studies are comparable with the current study, this may be due to the 10 day difference between the point in which the necropsies are conducted and when the current ewes gave birth. These studies support the conclusion that the organ weights of progeny are not directly affected by maternal nutritional status.

Backfat, a measure of carcass fat, was not quantified at birth due to the inability to accurately measure the limited fat that was present. For example, in some lambs no backfat was visible to measure. Tygesen et al. (2008) reported no difference in backfat depth for lambs from restricted-fed ewes or lambs born to over-fed ewes, but the lambs in the Tygesen et al. (2008) experiment were slaughtered at day 110 or day 146 of gestation. The duration of gestation measured in the current study and Tygesen et al. (2008) trial was similar, data was collected from the last third of gestation, as well from the lambs at the time of slaughter. At three months of age backfat was quantifiable and was reduced by 56% in restricted-fed ewes compared with control-fed ewes, and 50% less compared with overfed ewes at three months of age. Backfat was only quantifiable at three months of age due to the time needed for a measureable amount to accumulate (Long et al., 2011). The reduction in backfat thickness in the restricted born lambs correlates to changes in carcass quality which could negatively impact a production livestock situation. Meat quality and taste are linked to the amount of fat that is present both intra- and inter- muscular (Dransfield, 2008). Less fat creates a leaner muscle with a decrease in carcass quality, hurting the ability to market it to the consumer who associated fat content with tenderness and flavor (Dransfield, 2008).

As expected in conjunction with the decreased growth rate and body weight of the restricted-fed born lambs, IGF-I concentrations were decreased. Through the use of IGF-I knockout mice, it was reported that the obstruction of IGF-I signaling that there is 60% reduction in body weight at birth and 30% reduction in body weight at 8 weeks of age (Liu et al., 1993). Insulin-like growth factor-I is dependent upon the nutritional status of an animal and it has an essential role in the growth and development of the muscle, bone and organ systems (Hard et al., 2013). The 16% reduction in body weight of the restricted born lambs was likely the result of the 63% reduction in circulating IGF-I. Additionally, circulating IGF-I was reduced by 30% in lambs that were born to ewes that were overfed during gestation. These data support that maternal overnutrition and undernutrition have similar effects on IGF. Typically, increased IGF-I concentrations indicate enhanced protein accretion and an increase in lean muscle mass (Schwarz et al., 1993). Therefore, given the reduction in IGF-I, it was anticipated that there would have been a corresponding decrease in muscle mass. In contrast loin eye area, a measure of body muscle mass, in the restricted born lambs was comparable with both the control born lambs and overfed born lambs at birth. A possible explanation for the lack of difference in loin eye area may be due to the localized production of IGF-I; IGF-I is produced by all cell types including muscle cells (Hard et al., 2013). Therefore, although liver produced IGF-I is reduced due to poor maternal nutrition, cell specific IGF-I production which does not contribute to circulating concentrations could be unchanged.

Insulin-like growth factor binding protein-3, the main carrier of IGF-I, was decreased by 36% and 32%, respectively, in res-lambs and over-lambs compared with

con-lambs. Intrauterine growth retarded piglets, whose body weight was 67% less than littermates, had reduced IGFBP-3 at 90 days of gestation (Kampman et al., 1994). This reduction in IGFBP-3 during gestation appears to be in response to a decrease in the IGFBP-3 mRNA in the lambs (Kampman et al., 1994). Previously, in cattle that were fed 75% of nutritional requirements there was a 13% reduction in IGFBP-3 (Rausch et al., 2002). Although lambs in the current study were restricted secondary to the maternal target, similar effects were observed. This supports the importance of maternal plane of nutrition and its implications in the long term growth and development of the progeny. It has been shown previously that IGFBP-2 and IGFBP-3 are influenced by nutrition (Rausch et al., 2002). Current literature, and visual limitation of the western ligand blot, only IGFBP-2 and IGFBP-3 were quantified for the current study. Insulin-like growth factor binding proteins-3 and -2 were the only clearly visible to quantify through Opti-quant. The other four binding proteins were not clear on the gels or were too inconsistent for quantification using the current technique.

When animals experience nutritional restriction a disconnect occurs in the somatotropic axis. Normally GH and IGF-I are positively associated, whereas in nutritional restriction this association is uncoupled (Sosa et al., 2009). This uncoupling of the somatotropic axis involves increased GH concentrations, and decreased IGF-I concentrations (Sosa et al., 2009). In the current study, there was a decrease in circulating IGF-I in the lambs whose mothers were fed a restricted diet, therefore it was hypothesized that these lambs would have increased in circulating GH. There was no difference in circulating GH in the lambs born to restricted-fed ewes in the current study. Overfed born lambs compared with control-fed born lambs also had no difference in

circulating GH. The lack of difference reported may be the result of lack of statistical power in the analysis, due to limited serum and animals some time points had only a few data points. A greater number of data points would result in greater statistical power and stronger results.

The thyroid hormones, T3 and T4, were quantified at birth and three months of age in the current study. The hormones of the somatotropic and the thyroid hormones interact in a complex relationship, both GH and IGF-I has been reported to simulate the conversion of T4 to T3 (Juul et al., 2003). In the current study, there was no link observed between the concentrations of the somatotropic axis hormones and the thyroid hormones.

In summary poor maternal nutrition constitutes both underfeeding and overfeeding of an animal. The current study supports that underfeeding and overfeeding of a ewe had similar effects on the growth and development of lambs and altered the homeostasis of the somatotropic axis. Although there are similarities in the effects seen between the treatment groups there was a greater effect observed in the underfed group vs. the overfed group. Both maternal overnutrition and undernutrition resulted in a decrease in IGF-I and IGFBP-3. These decreases in the restricted-born lambs resulted in the reported decreased birth weight and decreased growth rate. In the overfed born lambs the decreases in circulating concentrations of IGF-I and IGFBP-3 resulted in a reduction in birth weight only at specific times. In conclusion, poor maternal nutrition, whether it be overnutrition or undernutrition, has permanent negative impacts on the growth and development of the progeny.

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